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Remote Controlled Capsules in Human Drug Absorption (HDA) Studies

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ABSTRACT: The biopharmaceutical complexity of today's new drug candidates provides significant challenges for pharmaceutical scientists in terms of both candidate selection and optimizing subsequent development strategy. In addition, life cycle management of marketed drugs has become an important income stream for pharmaceutical companies, but the selection of least risk/highest benefit strategies is far from simple. The proactive adoption of human drug absorption (HDA) studies using remote controlled capsules offers the pharmaceutical scientist significant guidance for planning a route through the maze of product development. This review examines the position of HDA studies in drug development, using a variety of case histories and an insightful update on remote controlled capsules to achieve site-specific delivery.

KEY WORDS: oral absorption, gastrointestinal tract, site-specific, gamma scintigraphy, intelligent capsules

I. INTRODUCTION

Advances in combinatorial chemistry, proteomics, and genomics have led to the potential for an unprecedented number of new molecular entities (NMEs) to exit discovery and enter full-scale development. Although the emphasis remains focused on developing oral products, few drug candidates have ideal biopharmaceutical properties for oral administration. Factors known to limit or inhibit drug absorption via the oral route include poor solubility or inadequate stability in gastrointestinal (GI) fluids, poor permeability across the intestinal epithelium, enzymatic or non-enzymatic degradation/metabolism in certain GI segments, and complexation with chelating ligands or metal cations normally present in the GI tract.[1]

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Pharmaceutical companies recognize the need to identify those compounds with problematic biopharmaceutical properties long before the first formal, prototype formulations are administered to humans.[2] As a consequence, drug discovery groups now routinely screen potential clinical candidates using a variety of in silico, in vitro and ex vivo technologies, as well as the more traditional live animal models. Although some of these technologies are suited to high throughput screening (HTS), output predictions are far from definitive and so need to be treated with caution. For example, the correlation between bioavailability in humans versus animals (rodent, dog, and primate) is surprisingly poor across a broad range of drugs (Fig. 1).[3]

Statistics recently presented by the US Food and Drug Administration (FDA) have further highlighted that a high proportion of drugs already reaching the marketplace are also likely to suffer from poor absorption. Since 1995, drugs categorized as Class 1 under the Biopharmaceutical Classification System (BCS) make up only 9% of New Drug Applications (NDAs). Class I compounds are defined as high solubility and high permeability, and so are predicted to be relatively well absorbed orally. All other compounds (Classes II–IV) suffer from low solubility, low permeability, or both; 46% of drugs approved since 1995 were BCS Class IV. Coupled with the fact that molecules well absorbed in the upper GI tract are often poorly

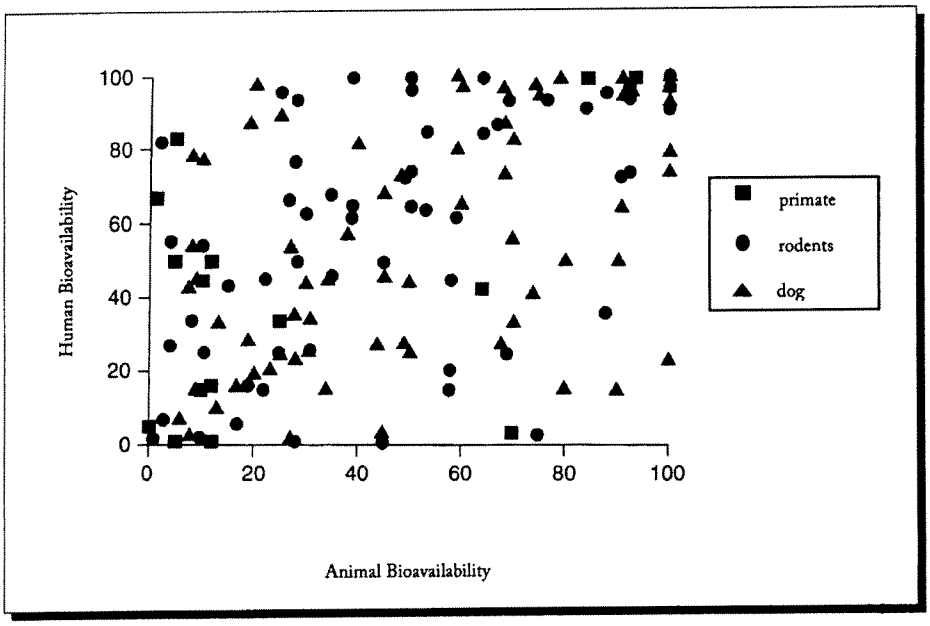

FIGURE 1. Absolute bioavailability in humans vs. animals.[3]

absorbed in the large bowel (or colon), these statistics imply that the application of conventional drug delivery approaches, such as modified release (MR), will become increasingly challenging, probably limiting future opportunities for life cycle management (LCM) of drugs approved within the last 5 or 10 years.

Increasingly, pharmaceutical and drug delivery companies are therefore using human drug absorption (HDA) studies to better understand the biopharmaceutical properties of early drug candidates and establish LCM strategies for marketed drugs. In addition, results from HDA studies undertaken early in clinical development give an indication of unsuitable or problem compounds and provide a reliable "route-map" for subsequent development.

The objective of this article is to review the current biopharmaceutical challenges in drug development in the context of using remote controlled capsules in the design and conduct of HDA studies.

II. DRUG ABSORPTION FROM THE GI TRACT

The GI tract is responsible for the extraction and absorption of nutrients from foodstuffs and so provides a natural pathway for the absorption of orally administered drugs.[4] The adult human small intestine is approximately 6 m in length and 3 to 5 cm in diameter. It is arbitrarily divided into three regions; the first 20–30 cm is the duodenum, the next 2.4 m is the jejunum, and the final 3.6 m is the ileum. The large intestine (or colon) is 3–9 cm in diameter and approximately 1.3 m in length. Striking differences can be seen in the surface area of the different regions: 1.5 m^2 for the colon compared to approximately 180 m^2 for the jejunum and 280 m^2 for the ileum. It is the effect of this high surface area that primarily accounts for increased absorptive capacity normally associated with the small intestine.

It is generally accepted that the unmediated permeation of molecules across the intestine occurs by two distinct pathways—paracellular and transcellular. Adjacent enterocytes (epithelial cells) making up the lipoidal epithelial layer are attached at the tight junctional complex near the cell apex. In the case of the paracellular route, molecules penetrate the tight junctions and pass through the epithelial cell and apical membrane to the underlying blood capillary. Paracellular permeation is thought to be maximal in the upper small intestine and limited in the colon.[4] The transcellular pathway is based on a consideration that the epithelium consists of a heteroporous barrier, allowing partitioning and diffusion of small molecules through the enterocyte membrane itself.

In addition to unmediated permeation, active transport mechanisms involving carrier and pump systems intrinsic to the cell membrane are also well recognized.[5] Carrier-mediated processes may be saturated or inhibited, and so may be regulated

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by a variety of internal or external factors. Saturability of carrier-mediated transport can lead to dose-dependent pharmacokinetics of drugs that are carrier substrates. Similarly, induction or inhibition of carriers may lead to enhanced or reduced absorption of drugs with an affinity for these carriers.

III. BARRIERS TO ORAL DRUG ABSORPTION

It is well known that for a drug to be absorbed, it must first dissolve in the intestinal fluid and then permeate across the epithelial layer. However, adequate aqueous solubility and permeability across a lipoidal membrane do not guarantee that the drug will be well absorbed. In the healthy, properly functioning intestine both efflux and metabolic processes occur at the gut wall to protect the body from exposure to noxious molecules.

III.A. Intestinal Efflux Systems

Several of the enzyme transport systems known to mediate efflux in the major clearing organs (e.g., liver and kidney) are also expressed in the intestine.[6] The most widely studied of these is P-glycoprotein (P-gp), a secretory transporter with broad specificity located on the mucosal surface of the epithelial cells. Drugs that are substrates for P-gp (or other multidrug-resistance-associated proteins) can be actively expelled from cells after entering via other routes. Selected drugs reported to interact with P-gp[7] are presented in Table 1. It is interesting that relatively hydrophobic drugs that would be expected to have a high permeability are also likely to be most susceptible to the P-gp efflux system. Coadministered drugs or other excipient materials that are substrates for P-gp may competitively inhibit the efflux system, which can lead to significant pharmacokinetic interactions.[8]

III.B. Intestinal Metabolism

The liver is the major metabolic organ, with cytochrome P450 identified as the primary catalyst responsible for oxidative metabolism. However, it is now recognized that there is also substantial P450 activity in the intestinal mucosa.[9] The majority of P450 found in the intestine is the isoform known as cytochrome P4503A4 (or CYP3A4), for which more drugs are known substrates than any other P450 subclass. CYP3A4 levels that have been found in the duodenum, jejunum, and ileum are presented in Table 2.[10] Studies have shown that the extent of so-called "gut-wall"

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TABLE 1. Selected Drugs Reported to Interact with p-Glycoprotein and/or Metabolized by Cytochrome 3A4

Drug Class	Substrate for p-Glycoprotein	Substrate for CYP3A4 and p-Glycoprotein	Substrate for CYP3A4
Antiarrhythmics	Propranolol	Amiodarone Lidocaine Quinidine	Propafenone Disopyramide
Antihistamines			Astemizole Loratadine Terfenadine
Antimicrobials	Cefoperazone Ceftrixone	Erythromycin Itraconazole	Dapsone Troleandomycin
Antiulcer			Omeprazole
Calcium Channel Blockers	Bepradil Tiapamil Nisoldipine Felodipine Nitrenidipine	Diltiazem Nifedipine Verapamil	
Hormones/Steroids	Aldosterone Deoxycorticosterone Clomiphene Dexamethasone Prednisone	Cortisol Progesterone Tamoxifen	Ethinyl estradiol Paclitaxel Testosterone
Immunosuppresents		Cyclosporine	Tacrolimus

metabolism can be significant, and a list of selected drugs metabolized by CYP3A4[11] is included in Table 1. However, interactions are complex, and certain drugs (such as rifampin) may induce the activity of CYP3A4, whereas others (such as ketoconazole) inhibit the enzyme system.[12,13] From a drug development perspective, the

TABLE 2. Levels of Cytochrome P450 3A4 in the Human Intestine[10]

Intestinal region	Length (approx)	Total CYP3A4	Variation in CYP3A4
Duodenum	0.2 m	9.7 nmol	3–90 pmol/mg
Jejunum	2.4 m	38.4 nmol	2–98 pmol/mg
Ileum	3.6 m	22.4 nmol	2–38 pmol/mg

overlap of compounds that are subject to both P-gp efflux and CYP3A4 metabolism is striking. If both these systems could be selectively and reversibly inhibited, the bioavailability of many poorly absorbed drugs could be significantly enhanced. Table 1 identifies a partial listing of such "problem drugs" whose bioavailability is limited by both mechanisms.

III.C. Degradation by Colonic Bacteria

The large intestine normally contains large numbers of bacteria (natural microflora) such as *Bifidobacterium, Bacteroides, Eubacterium,* and *Peptostreptococcus.* These serve to control colonic pH, aid digestion, stimulate the immune system, and inhibit the growth of other food-poisoning or disease-causing bacteria.[14] Some drugs, such as ranitidine,[4] are susceptible to degradation by colonic bacteria and tend to show reduced bioavailability from the colon.

IV. REMOTE CONTROLLED CAPSULE DEVICES

Historically, the most popular approach for determining the absorption of drugs from different regions of the intestine has been through the use of perfusion or intubation techniques.[15-17] These techniques require invasive tubes to be placed at the relevant part of the GI tract via the mouth or rectum. Once located at the correct region, a drug solution or suspension is infused into the gut lumen at a pre-determined rate. Such invasive procedures are obviously associated with significant volunteer discomfort, and, more important, the presence of a tube in the intestines has been demonstrated to alter the function of the GI tract.[18] In particular, intubation has been shown to influence the absorption and secretion balance within the gut, which calls into question the pharmaceutical relevance of drug absorption data collected via this approach.

The concept of using swallowable, remote controlled capsules to avoid any GI disruption often associated with invasive techniques is not new. Back in 1960, Eriksen et al.[19] constructed what we believe to be the first remote controlled capsule specifically for the purpose of performing regional drug absorption studies. Although the design was relatively crude by today's standards, the device was reported suitable for the delivery of both solid and liquid formulations. The capsule consisted of an Inconel® (nickel/chromium alloy) tube that was sealed using wax at both ends. It was activated using a 500-kHz radio frequency generator, which caused a slow warming of the metal tube, eventually softening the wax. Once the metal warmed to above 50 °C, a spring was released, which served to eject the solid capsule contents

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via a pusher acting on either a brass frame containing the drug powder or tablet form. In the case of liquid formulations, the forward motion of the spring-driven pusher forced a hole in the rear wax seal, allowing the liquid contents to drain out. The position of the capsule in the GI tract at the time of drug release was originally assessed using a trailing length of Dacron sewing thread, although fluoroscopic measurements were later substituted. The methodology described in that study was claimed suitable for human studies, although to our knowledge data was only ever published for the absorption of aqueous sodium salicyliate in dogs.[19]

Over the last 20 years a number of other remote controlled capsule devices have been developed and sold commercially to allow site-specific measurement of human absorption in a noninvasive manner.[19] The primary emphasis of these different systems has been the ability to control the time and location of drug release. Below is a summary of the operative mechanisms for the most important and widely used of these technologies.

IV.A. High-Frequency (HF) Capsule

The HF capsule (Battelle-Institute V, Frankfurt am Main, Germany) was developed in the early 1980s. After being swallowed, the passage of the capsule is tracked using X-ray fluoroscopy. On reaching the target location, activation is triggered by a radio-frequency (RF) pulse from a high-frequency generator external to the body. Heat generated as a result of the RF-induced current melts a thread and releases a needle, which in turn pierces a latex balloon and allows drug to passively empty from ports in the wall of the capsule.

The HF capsule has been used successfully to study the regional absorption of a variety of drugs, such as ipsapirone[20] and nifedipine.[21] However, this device is not particularly well suited to delivery of particulate formulations because of its passive release mechanism and difficulty of filling powders into the balloon. Furthermore, the use of X-ray fluoroscopy to track the location of the capsule has further limited its application because of the potentially high radiation dose arising from studies targeting colonic absorption.

IV.B. Gastrotarget Capsule

The Gastrotarget telemetric capsule (Gastrotarget Corporation, Tonawanda, New York) has a relatively complicated mode of action. An external RF pulse triggers activation by generating heat in the capsule that releases a membrane. A needle then punctures the membrane, causing reagents to mix and liberating carbon dioxide gas. The buildup in pressure moves a piston, which in turn expels a plug releasing the

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200 μL contents. Upon activation, an indicator signal is detected externally and so provides confirmation of in vivo delivery. Location of the capsule in the GI tract is assessed by sending a dummy capsule through the GI tract of the subject 24 hours prior to the actual study day. This limits the reliability and reproducibility of the technology as well as adding further cost and subject inconvenience.

IV.C. Telemetric Capsule

The Telemetric capsule (INSERM U161, Strasbourg, France) comprises a location detector, transmitter, lithium battery, and interchangeable drug reservoir tip (Fig. 2). The capsule is activated by application of an external magnet to trigger a magnetic switch. This operates a battery-powered micro-furnace, which in turn breaks a plastic strip, releasing a compressed spring. Movement of the spring opens an aspiration orifice through which the pressurized drug contents are expelled. Location of the capsule within the bowel is assessed by rotation of cog wheel, whereby the data are transmitted by the capsule and interpreted by the investigator. This mechanism has the advantage of being radioactive-free, but is prone to error, given the normal inter-subject variability in intestinal anatomy. Furthermore, the device has a high current drain, limiting battery life to about 8 hours, although this may now be extended with recent advances in battery technology. Other disadvantages of the Telemetric capsule include the potential for prolonged stomach retention as a result of its large physical size and the narrow release orifice, which generally makes it unsuitable for particulate delivery.

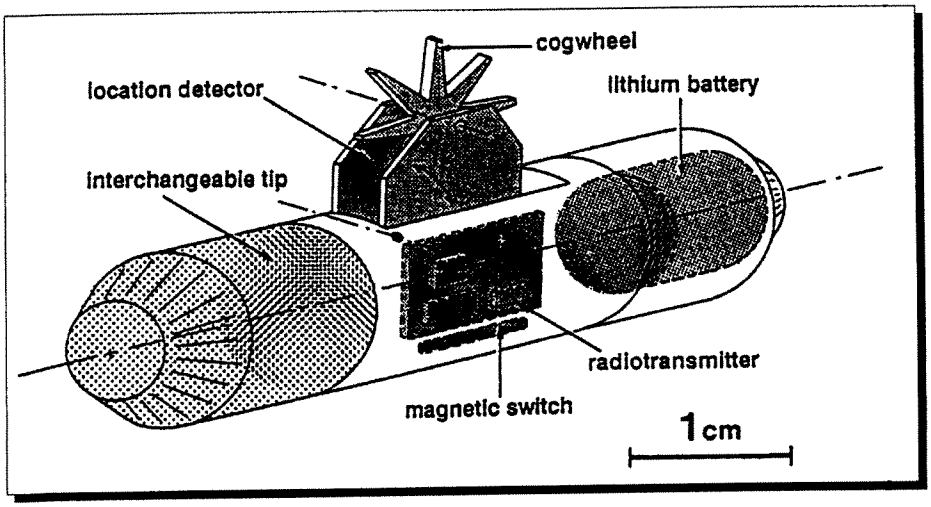

FIGURE 2. The Telemetric capsule.

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IV.D. InteliSite Capsule

The InteliSite capsule[22] (Innovative Devices, Raleigh, North Carolina) was commercialized in the late 1990s. At only 10 mm in diameter and 35 mm in length, the InteliSite is more compact than its predecessors. It consists of an on-board electronics/actuator assembly, a drug reservoir 0.8 mL in volume, and a radiotracer port (Fig. 3). Location in the GI tract is followed using a gamma camera by placing a short half-life, gamma-emitting radionuclide inside the sealed radiotracer port.

The capsule is activated by application of an external oscillating magnetic field, which induces an electric current in a 3-dimensional array of receiving coils. This slowly warms a contact plate, transferring heat to a pair of shape memory alloy (SMA) wires. As these wires straighten, the inner sleeve rotates to align a series of apertures

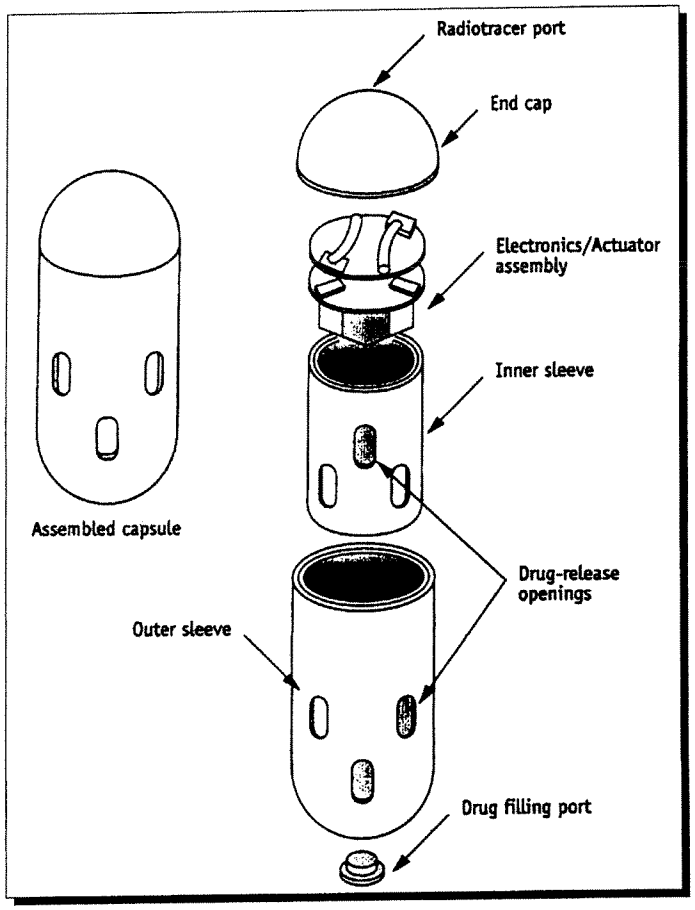

FIGURE 3. The InteliSite capsule.

with corresponding apertures in the outer sleeve. Alignment of the apertures allows passive release of drug from the reservoir.

As with other passive release systems, the lack of free water and agitation does not favor complete and reproducible delivery of particulate formulations to the distal colon, which has compromised the reliable use of InteliSite[23,24] in commercial studies. Other difficulties encountered with InteliSite include slow or possibly failed activations if the capsule is particularly deep in the body, as well as the potential for preactivation leakage from the drug reservoir.[24]

IV.E. Enterion Capsule

The Enterion capsule (Phaeton Research, Nottingham, UK) is the latest advance in available HDA technologies and overcomes the primary limitations of the earlier devices.[25] It is a round-ended capsule, 32 mm in length and 11 mm in diameter, with a drug reservoir of approximately 1 mL in volume located within the main body (Fig. 4). The active delivery mechanism makes this technology extremely versatile and fully effective, with a wide variety of formulations, including solutions, viscous suspensions, particulates, pellets, and minitablets.

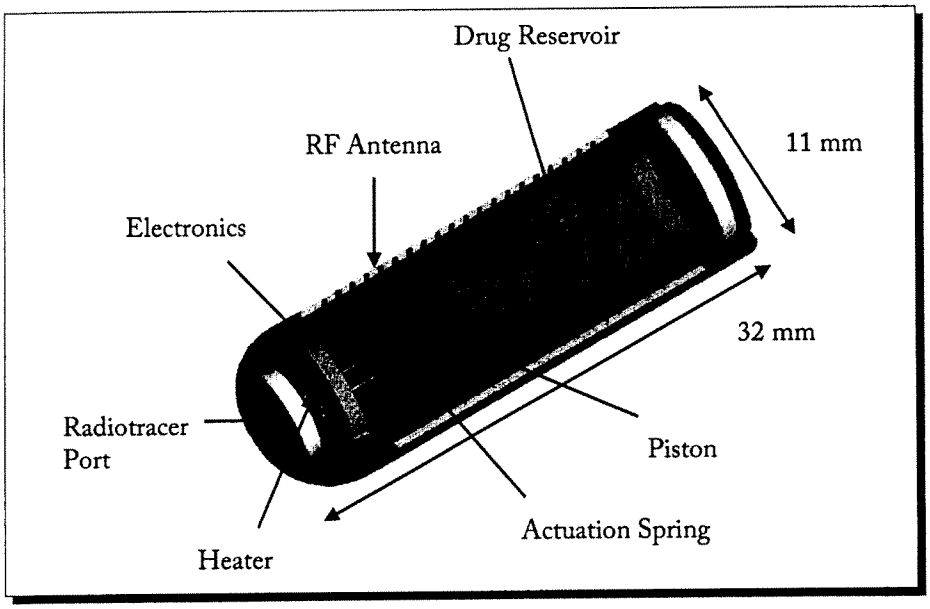

FIGURE 4. The Enterion capsule.

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The capsule is loaded with the drug (or drug formulation) through an opening 9 mm in diameter, which is then sealed by inserting a push-on cap with a silicone O-ring gasket. The floor of the drug chamber is a piston face, which is held back against a compressed actuation spring by a high tensile strength polymer filament (the spring latch). To track capsule location after administration, a radioactive marker is sealed inside a separate radiotracer port beneath the rounded end cap. This allows capsule position to be followed in real time using a gamma camera.

When the capsule arrives at the target site in the GI tract, it is remotely triggered by application of an oscillating electromagnetic field, which is generated over the abdominal cavity by an external radio-frequency (RF) generator. The frequency of the field is 1.8 MHz, which is low enough for negligible absorption of energy by the body tissues, but sufficiently high to induce usable power in a tuned RF antenna embedded inside the wall of the capsule. The electric current induced by the magnetic field in the receiving coil is fed to a low-power (0.0625 W) heater resistor, which is situated within a sealed electronics compartment. The small size of the heater (<1 mm^3) results in a rapid temperature rise in just a few seconds.

The spring latch filament that anchors the piston is in direct contact with the heater. As rapid heat buildup occurs, the filament quickly reaches a critical temperature, at which point it softens and immediately breaks under the tensile strain of the spring. The energy stored in the spring is only about 0.18 joules; however, the relatively low mass and friction coefficient produces high acceleration of the piston. Once the spring is released, it drives the piston into the drug chamber, forcing off the O-ring sealed cap (this force is rapidly dissipated as the cap quickly decelerates in the relatively viscous GI lumenal fluids). Under the continued forward motion of the spring-driven piston, the entire capsule contents are actively expelled into the surrounding GI environment within milliseconds. A restraining (or stop) ring situated near the end of the capsule stops the piston movement. This also maintains the seal and so prevents contact of the electronic components with GI fluids.

As the piston travels the first centimeter immediately after activation, it operates a switch that diverts the incoming electrical energy from the heater resistor to a radio-frequency transmitter coil (also embedded inside the capsule wall). This generates a weak radio signal at approximately 500 kHz, which is picked up by an external aerial. Detection of the radio signal confirms that the capsule has opened successfully and is used to initiate the blood sampling protocol.

The Enterion capsule was specifically designed to ensure the reliable delivery of both liquid and particulate formulations, especially to the distal colon, where there is minimal free water[26] to assist passive drug delivery. The volume ratio of the drug chamber to the overall capsule size was also an important consideration in providing maximum versatility while ensuring that subjects could swallow the capsule relatively easily without any serious discomfort or gag reflex. A summary of the essential attributes and design features of the Enterion capsule is presented in Table 3.

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TABLE 3. Key Design Features of the Enterion Capsule

Essential attribute	Enterion design feature
Biocompatible	Food contact and medical grade plastics used for fabrication of all parts that come into contact with GI-lumenal fluids.
Readily swallowed by volunteers	Round-ended with overall dimensions (32 mm × 11 mm in diameter) comparable to 000-sized gelatin capsule
Easy tracking of capsule location	Short half-life radionuclide sealed inside a radiotracer port allows tracking via a gamma camera.
Suitable for delivering a range of physical forms	Spring-driven piston ensures rapid and complete delivery of particulate, semi-solid, and liquid formulations.
High loading capacity	Drug chamber approximately 1 mL in volume
No drug leakage prior to activation	Compressed silicone O-ring provides a reliable closure system with high seal integrity.
Reliable activation at all intestinal sites	Compressed spring provides an on-board energy source. RF activation frequency selected to avoid absorption by human tissue. Proprietary cap release mechanism based on a unique "rolling" O-ring design.
Feedback signal to confirm drug delivery	RF signal generated on forward motion of the piston.

An in-depth technical description of the Enterion capsule is provided in two published patent applications, which embody both the capsule[27] and the unique features of the cap release mechanism.[28]

V. APPLICATION OF HDA STUDIES

V.A. Development of New Molecular Entities (NMEs)

Today, product development scientists are regularly faced with complex compounds exiting drug discovery as lead clinical candidates. Many compounds exhibit one or more of the following undesirable biopharmaceutical properties, which limits the intrinsic bioavailability:

- poor aqueous solubility
- instability in gastrointestinal fluids

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- complexation with chelating ligands or metal ions present in the GI tract
- poor permeability across the intestinal epithelium
- narrow absorption window in the upper small bowel
- intestinal efflux
- gut-wall metabolism

Early characterization and understanding of these properties can assist the development scientist in the selection of the right enabling (drug delivery) technology and thereby accelerate development of the optimal dosage form. Examples of where HDA studies can be best applied according to a drug's physiochemical properties and development objectives are presented next.

1. Poor Aqueous Solubility

Over 40% of the NMEs currently in development are estimated to have poor aqueous solubility.[29] A host of traditional, as well as proprietary, drug delivery technologies are available to improve the bioavailability of such compounds. Generally, these enabling technologies involve either the classical approach of reducing the drug particle size (e.g., production of stabilized nanoparticles) to enhance dissolution rate, or else use nonaqueous solvents and/or surfactants to enhance intrinsic in vivo solubility through the creation of a solution or microemulsion.[29]

HDA studies are particularly valuable for discriminating between drugs with solubility or permeability-limited absorption. A common study design involves delivery of the drug to one or more intestinal regions in two different forms, a particulate form (such as a powder or granule) and a solution (or other solubility-enhanced) form. If the solution exhibits a significantly higher bioavailability at one or more of the absorption sites, then solubility (i.e., dissolution of the drug in lumenal fluids) is likely to be the limiting factor. Alternatively, if bioavailability is essentially unaffected by the form of the drug, then absorption must be permeability limited.

Anecdotal evidence suggests that the number of compounds with suboptimal aqueous solubility will continue to increase as the industry targets compounds with higher pharmacological activity, potency, selectivity, and specificity.[29] This dilemma may ultimately be overcome through closer cooperation between discovery chemists and development scientists, but in the meantime selection of the enabling technology best suited to the compound in question will remain at the forefront of successful product development. To this end, HDA studies can play an important role. Measurement of drug absorption in key segments of the GI tract and then comparing these

baseline data to the same drug in a solubility- or dissolution-enhanced formulation can provide valuable data on the maximum threshold of oral bioavailability.

2. Poor Intestinal Permeability

The biotechnology revolution is providing a growing number of peptides, proteins, and genomic drug candidates. These macromolecules are often rapidly degraded in the GI tract and almost always suffer from low intestinal permeability. Achieving acceptable oral dosage forms of such compounds is therefore far from straightforward.

Enabling technologies that may overcome the problem of poor permeability include formulation with custom-synthesized carriers, use of chemical enhancer systems to alter transcellular absorption, and even techniques specifically altering the tight junctions between cells to facilitate paracellular uptake.[30] Although the potential rewards are great, these approaches may be fraught with significant regulatory obstacles because the potential adverse effects of altering the permeability of the intestinal barrier are not yet fully researched.

HDA studies are a valuable tool for determining the oral bioavailability of biotechnology compounds and assessing the performance of potential permeability enhancers. Using a remotely triggered device such as the Enterion capsule, problems caused by gastric instability are overcome by delivering the drug (or drug formulation) directly to the most favorable sites of absorption. This allows rapid and reliable screening of the clinical candidate (with or without any companion delivery technology), which can accelerate key development decisions. For example, a compound suffering a negative human absorption finding could be deemphasized from development, returned to discovery for reengineering, or evaluated for alternative routes of delivery such as pulmonary, nasal, or parenteral.

3. Intestinal Metabolism and Efflux

An increasing number of NMEs demonstrate not only complex chemistry, but also low and highly variable pharmacokinetics. CYP3A4 gut-wall metabolism in combination with intestinal transporter systems can provide major development obstacles for NMEs.[31] As more transporter systems are discovered, a growing number of companies are attempting to develop technologies designed to inhibit intestinal metabolism and/or efflux. HDA studies can be used to evaluate the absorption of drugs susceptible to these enzyme systems and directly measure the performance of codelivered permeation enhancers intended to improve bioavailability.

Targeted drug delivery may also be used to overcome the inhibitory effects of intestinal metabolism and efflux. In a recent HDA study,[32] a drug with low

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TABLE 4. Site-Dependent Metabolism—Relative Bioavailability (AUC) vs. Control [CV%]

	AUC versus Control
Proximal small bowel	70% [25]
Distal small bowel	89% [18]
Ascending colon	113% [14]

oral bioavailability, highly variable pharmacokinetics, and a known substrate for CYP3A4 was delivered to the proximal small intestine, distal small intestine, and ascending colon. Relative bioavailability (AUC) was measured versus an immediate release reference in a fully randomized, four-way crossover study in eight healthy volunteers. The drug was found to exhibit a higher and less variable bioavailability when delivered to the ascending colon (Table 4). These results prompted the pharmaceutical company to fast-track development of a modified release formulation and seriously consider parallel development of a colon-targeting formulation to maximize drug absorption.

4. Narrow Absorption Window

The anatomy and physiology of the human intestine means that relatively few drugs are well absorbed throughout the entire GI tract. Indeed, the overwhelming majority show decreasing permeability on descending from the proximal small intestine to the large bowel. Compounds such as L-dopa and ciprofloxacin are classic examples of drugs that exhibit a narrow absorption window in the upper intestine, making development of sustained release formulations extremely challenging.

Several drug delivery companies are developing enabling technologies based on the concept of gastric retention,[29] including rapid swelling/slow erosion formulations or multiparticulate, mucoadhesive systems. HDA studies are invaluable for compounds vulnerable to absorption window effects and can provide fundamental data for selecting the optimal enabling technology.

V.B. Life Cycle Management (LCM) of Established Drugs

Pharmaceutical companies continue to search for more creative line extensions as they strive to maximize revenue from existing marketed drugs. For oral products, the LCM strategy often involves development of an MR dosage form designed to

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offer clinical, safety, or compliance benefits. Examples include extended release (ER) technologies, targeted intestinal approaches, or chronotherapeutic delivery systems. Knowing the rate and extent of drug absorption from different regions of the GI tract is essential to the rational and cost-effective development of such products.

The traditional approach for developing an MR delivery system begins with the preparation and in vitro dissolution testing of early formulation prototypes to predict the possible drug delivery characteristics in vivo. Once acceptable laboratory data are obtained, the pharmaceutical company will typically undertake a straightforward pharmacokinetic study to evaluate actual in vivo performance. It is not unusual for the outcome of such studies to be equivocal, necessitating changes to the formulation without a clear indication of how (or even if) drug delivery can be optimized to achieve the development objectives. Modified prototypes are then often evaluated in a series of pharmacokinetic studies, such that the formulation is gradually refined through an iterative process (or until the development is eventually deemed unviable).

A more rational and cost effective approach is to first conduct a straightforward HDA study. Measurement of drug absorption from the key residence regions defines the "art of the achievable" from the outset, thereby enabling informed decision making before valuable development resources are spent in the preparation and testing of complex formulation prototypes. Example applications of HDA studies that have assisted the development of oral MR products are presented next.

1. Extended (or Sustained) Release

The total gastrointestinal transit time for an extended release (ER) product is about 24 hours. Typical residence times in each of the key regions of the human GI tract

TABLE 5. Residence Times in Key Regions of the Human Gastrointestinal Tract Following Fasted Dosing of a Tablet Formulation[a]

Region	Residence Time (hrs)		
	Mean	Range	Cumulative Mean
Stomach	0.5	0–2	0.5
Jejenum	1.25	0.5–2	1.75
Ileum	1.5	0.5–2.5	3.25
ICJ	1.25	0–12	4.5
Colon	20.0	0–72	24.5

[a] Pooled data from human studies 1985 to present day.

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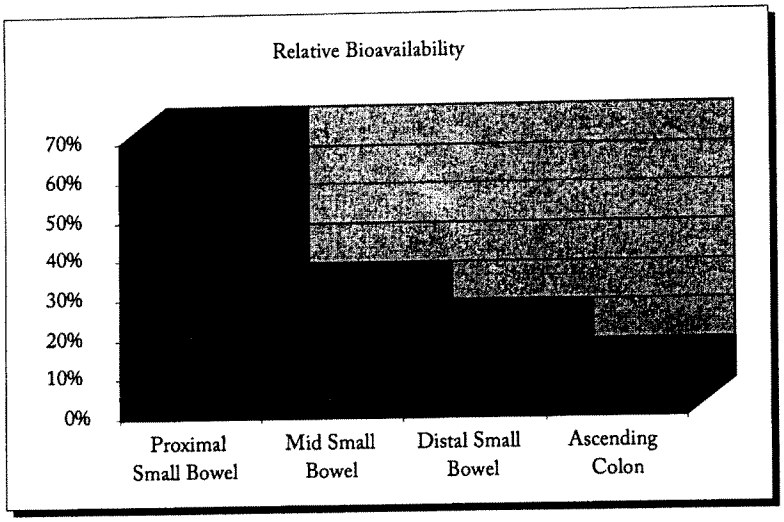

FIGURE 5. Bioavailability of a proprietary drug in different regions of the human gastrointestinal tract relative to an oral solution (n = 10 healthy volunteers).

are presented in Table 5. This shows that approximately 80% of transit time is normally spent in the colon, with only around 15% in the small intestine.[4] It is therefore essential to have an understanding of drug absorption from the key regions of the human gastrointestinal tract (e.g., proximal jejunum, terminal ileum, and colon) before commencing any ER development strategy.

Results from a recent drug absorption study are provided in Figure 5.[33] A proprietary drug was selectively delivered to four target sites (proximal small bowel, mid small bowel, distal small bowel, and ascending colon). Bioavailability was demonstrated to be highly dependent on site of delivery in a randomized, five-way crossover study in ten healthy volunteers versus an oral solution reference. Most critical, drug absorption was poor in the distal intestines, which would severely inhibit development of a once-daily product using conventional ER technologies. The availability of human absorption data therefore allowed early definition of possible development strategies.

2. Chronotherapeutic Drug Delivery Systems

The recent emergence of chronotherapeutic delivery is demanding ever more sophisticated MR technologies that can control variable drug input rates sympathetic to the

body's natural biological rhythms. These technologies often involve a combination of delayed, extended, and pulsed release of the drug.

In a recent HDA study,[32] a drug exhibiting rapid and complete oral absorption was screened as a candidate for pulsed release. The drug (low solubility/high permeability or Class II, according to the BCS) had previously shown good colonic permeability in both the rat and dog preclinical models. However, using the Enterion capsule for targeted delivery in humans, only about 30% relative bioavailability (compared to IR) was found in both the ascending and descending colon segments. As a result of these findings, the pharmaceutical company abandoned the pulsed-release development and recognized that the earlier preclinical data had not been predictive of human absorption.

3. Development of a Novel Salt or Prodrug

Switching a drug to a different salt form or new prodrug prior to patent expiry is a tactic sometimes employed as part of an overall LCM strategy. An HDA study can quickly and conveniently confirm that any new form retains (or has improved upon) the biopharmaceutical properties of the original molecule before embarking upon the costly pharmaceutical development.

VI. HDA CASE STUDY EXAMPLES

From launch of the Enterion technology in March 2000 to September 2003, over 1000 capsules have been dosed and activated successfully to approximately 400 individual subjects. Most HDA studies are sponsored by major pharmaceutical companies and involve proprietary, early phase compounds; hence opportunities to publish detailed findings are rare. However, the results of several absorption studies have recently entered the public domain.

VI.A Faropenem Daloxate[33]

Faropenem Daloxate (FD) is a novel ester prodrug of faropenem sodium, a synthetic broad-spectrum oral antibiotic. After oral administration, FD is rapidly absorbed and hydrolyzed in serum to the active moiety, faropenem (FAR). The study was designed to compare the bioavailability of FD when delivered to the proximal small bowel (PSB), distal small bowel (DSB), and ascending colon (AC) versus the immediate release (IR) tablet. A single dose (equivalent to 300 mg FAR) was administered in a

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TABLE 6. Pharmacokinetic Parameters of Faropenem (Free Acid) Following Targeted Delivery of Faropenem Daloxate in Particulate Form Using the Enterion Capsule

PK Parameter (free acid)	IR tablet formulation	Proximal small intestine	Distal small intestine	Ascending colon
AUC (mg.hr/L)[a]	25.80 (1.12)	22.70 (1.17)	20.13 (1.17)	8.64 (1.72)
Cmax	15.30 (1.44)	11.79 (1.25)	9.96 (1.21)	2.29 (1.65)

[a] Geometric mean (geometric SD)

randomized, four-way crossover study in eight healthy male subjects. The Enterion capsule was loaded with a powder formulation (crushed IR tablet). To further assist with real-time interpretation of each subject's GI anatomy, the water used to administer the Enterion capsule was radiolabeled with ^{99m}Tc-DTPA solution (4 MBq). Following confirmation of capsule activation, blood samples were taken over 24 hours and subsequently analyzed using a validated HPLC method with UV detection.

The PK profiles following delivery to the PSB and DSB were similar and comparable to the IR reference tablet (Table 6). The relative bioavailabilities (AUC) were 87% and 80%, respectively. Significant colonic absorption was also demonstrated for all subjects following delivery to the AC; however, AUC and C_{max} were markedly reduced to 31% and 15%, respectively. The study sponsor considered these results essential in predicting the optimal MR profile for FD and therefore extremely useful in guiding future product development.

VI.B Oseltamivir[34]

Oseltamivir is an orally available ester prodrug of oseltamivir carboxylate and is approved as a 75-mg twice daily regimen for the treatment of influenza A and B. Oseltamivir is metabolized in the liver with a high hepatic extraction ratio, and about 80% of an orally administered dose of oseltamivir reaches the systemic circulation as the active carboxylate metabolite.[35] The prodrug is fairly polar, while the carboxylate form is highly polar (log P of 0.36 and −2.1, respectively). Oseltamivir has a high aqueous solubility (>500 mg/mL) and moderately low Caco-2 permeability coefficient ($1.2 \pm 2.2 \times 10^{-5}$ cm/s). Therefore, as a BCS class III drug, its absorption could be variable and intestinal-site dependent. The purpose of this study was to investigate the effect of intestinal site on the rate and extent of oseltamivir absorption using a site-specific delivery technology and to consider the feasibility for MR formulation development for once daily administration.

Oseltamivir (150 mg) was delivered to a group of nine subjects via the Enterion

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TABLE 7. In Vivo Activation Time (Post-Dose) and Anatomical Location for the Site-Specific Delivery Capsule (Mean ± SD [h])

Target activation site	Activation time	Activation site
Stomach	0.11 ± 0.07	100% stomach
Proximal small bowel	2.27 ± 1.46	100% jejenum
Distal small bowel	2.66 ± 0.63	100% terminal ileum
Ascending colon	6.08 ± 1.58	100% ascending colon

TABLE 8. Oseltamivir and Carboxylate Pharmacokinetic Parameters (Mean (CV%)) After 150 mg Oseltamivir Released in the Proximal Small Bowel, Distal Small Bowel, and Ascending Colon Compared with Release in the Stomach

Parameter	Stomach	Proximal small bowel	Distal small bowel	Ascending colon
Oseltamivir				
Cmax (ng/mL)	99.5 (21.8)	104 (41.7)	132 (42.1)	48.6 (65.5)
$AUC_{0-\infty}$ (ng*h/mL)	189 (23.1)	176 (23.4)	178 (36.0)	157 (33.8)
Tmax (h)	0.50 (0.0)	0.61 (36.1)	0.56 (30.0)	0.84 (51.9)
t1/2 (h)	1.6 (41.8)	1.3 (13.5)	1.4 (24.3)	6.6 (82.8)
Carboxylate				
Cmax (ng/mL)	487 (19.9)	463 (19.6)	442 (38.7)	197 (39.2)
$AUC_{0-\infty}$ (ng*h/mL)	5660 (19.4)	5660 (18.8)	5040 (25.1)	3830 (23.4)
Tmax (h)	2.9 (20.8)	3.9 (23.9)	3.2 (13.7)	5.9 (36.5)
$t^1/_2$ (h)	6.5 (19.2)	6.8 (14.8)	6.6 (15.0)	9.8 (30.9)
Metabolite/parent ratio	34.0 (23.6%)	36.5 (24.3%)	33.0 (23.7%)	29.5 (34.1%)

capsule to four separate specific sites along the GI tract: the stomach (reference site), the proximal small bowel (jejunum), the distal small bowel (ileum), and the ascending colon. GI location of the capsule was monitored in real time by gamma scintigraphy.

In vivo activation times of the Enterion capsules and the anatomical location of drug release are listed in Table 7. In general, the pharmacokinetics of oseltamivir and its carboxylate were similar for the proximal and distal small bowel, compared to those for the stomach, while the parameters for the ascending colon were reduced (Table 8). Rate and extent of absorption following delivery to the proximal small bowel and to the distal small bowel were bioequivalent to delivery to the stomach. Following colonic delivery, the $AUC_{0-\infty}$ for parent drug and metabolite were reduced to 83% and 68% compared to stomach.

Therefore, the selection of delivery systems that release oseltamivir in any region of the small intestine will have minimal effect on absorption and metabolism of oseltamivir, while colonic delivery may result in reduced absorption. However, the residual colonic absorption is nevertheless substantial and could supplement small bowel absorption.

It is interesting that oseltamivir is a reasonably polar drug, which suggests the potential for paracellular absorption to play a significant role in drug uptake. However, previous studies have shown reduced colonic absorption for polar drugs because of the low porosity of the tight junctions in the large bowel.[36] The good absorption of oseltamivir in the colon therefore suggests a mixed absorption mechanism with significant opportunity for transcellular uptake despite the relatively polar properties of the prodrug.

VI.C. Lumiracoxib[37]

Lumiracoxib is a novel cyclooxygenase-2 (COX-2) selective inhibitor (mw: 294 D; pKa: 4.7; logP: 1.2; water solubility: 0.03 mg/mL), which has been developed as an oral formulation for the treatment of the signs and symptoms of osteoarthritis and rheumatoid arthritis and the management of acute pain. Single dose studies using an IR formulation indicate that lumiracoxib is rapidly absorbed with a T_{max} of 1–4 hours post-dose and a relatively short plasma half-life of 3–6 hours. The aim of this study in eleven healthy volunteers was to assess the bioavailability profile of lumiracoxib following delivery at specific sites along the GI tract (stomach, proximal small bowel, distal small bowel, and ascending colon) using a remote-controlled capsule in order to provide insights into the pharmacokinetic characteristics of lumiracoxib in an IR form.

Plasma concentration versus time (post-dose) profiles for each of the targeted sites of capsule activation are shown in Figure 6. In general, plasma concentrations of lumiracoxib were quantifiable up to 24 hours post-dose. Following release of lumiracoxib in the stomach, a mean C_{max} of 1390 ng/mL was achieved 2 hours post-dose (Table 9). Mean AUC_{0-t} and $AUC_{0-\infty}$ following release of lumiracoxib in the proximal or distal small bowel were similar to those observed following release in the stomach, although in both cases C_{max} was higher (Table 9). Release of lumiracoxib in the ascending colon resulted in only a slightly lower AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} than in the other three regions. Mean T_{max} following capsule release in the stomach was longer than seen for capsules released in the small bowel or colon.

Statistical comparisons of AUC parameters showed only slightly decreased bioavailability following drug release in the ascending colon relative to that following drug release in the stomach. Bioavailability of lumiracoxib following capsule activation in the stomach and small bowel were similar. C_{max} appeared to be ap-

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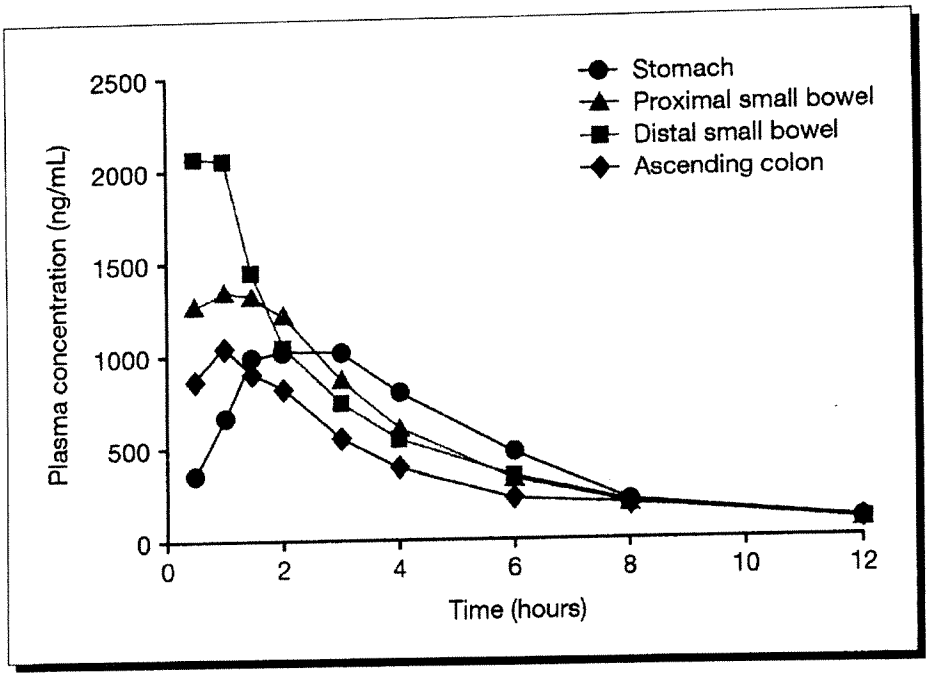

FIGURE 6. Mean plasma concentrations of lumiracoxib following regional delivery in the GI tract over 12 hours.

TABLE 9. Key Pharmacokinetic Parameters Following Regional Delivery of Lumiracoxib in the GI Tract

	Mean ± SD			Median (range)
Site of Activation	$AUC_{0-\infty}$ ng•h/mL	$AUC_{0-\infty}$ ng•h/mL	C_{max} (ng/mL)	T_{max} (h)
Stomach	6146 ± 944	6011 ± 940	1390 ± 513	2.0 (1.0–6.0)
Proximal small bowel	6603 ± 2186	6355 ± 2321	1628 ± 663	1.0 (0.5–2.0)
Distal small bowel	6842 ± 1473	6691 ± 1525	2413 ± 996*	1.0 (0.5–1.0)
Ascending colon	4983 ± 1145	4736 ± 1144	1109 ± 612	1.0 (0.5–2.0)

* statistically different

proximately 1.5 times higher in the distal small bowel than in the stomach, and T_{max} occurred approximately 1.5 hours later in the stomach than in the small bowel and ascending colon.

In contrast with other COX-2 selective inhibitors, lumiracoxib is a weak acid (pKa: 4.7), which results in limited solubility in the stomach fluids (<0.01 mg/mL at pH 1.2 SGF and 0.25 mg/mL at pH 6.8 SIF). The drug was dosed as a PEG 4000 solution, and it is probable that following release in the stomach, drug crystallizes in the gastric contents as a result of the low aqueous solubility at acidic pH. However, on gastric emptying, the crystallized drug quickly redissolved as a result of the enhanced pH properties of the jejunum. This may explain the longer T_{max} observed for lumiracoxib released in the stomach than in the small bowel and ascending colon (Table 9). These findings suggest that lumiracoxib more rapidly dissolves in the basic environment of intestines, leading to an increased absorption rate, as demonstrated by the improvement in C_{max}. However, it is interesting to speculate on the differences in C_{max} between PSB and DSB delivery. There are subtle but significant differences in the pH of these intestinal sites; PSB (jejunum) is likely to have a pH of around 6.5, while the DSB (terminal ileum) is about one pH unit higher, at 7.5.[4] The solubility of lumiracoxib at the pH of the DSB is approximately 8 times greater than in the PSB and presumably minimizes the possibility of any crystallization following delivery into the latter regions of the small bowel, which enhances the C_{max}.

Overall, the results indicate that lumiracoxib is rapidly and efficiently absorbed by the GI tract and has no specific window for absorption. This provides an opportunity for rational development of IR formulations as well as alternative dosage forms.

VII. WHEN TO PERFORM AN HDA STUDY

It might be argued that the majority of orally active compounds identified as clinical candidates or designated for LCM should be routinely screened by performing an up-front proactive HDA study. However, it is more likely that the decision to perform an HDA study will be taken on a case by case basis, at least for the foreseeable future. Compounds likely to need any form of oral MR technology are obvious candidates for which an HDA study should become an integral part of development pharmaceutics.

For compounds in early development, a particularly cost-effective approach is to combine the measurement of regional bioavailability with one of the more standard PK studies, such as absolute bioavailability or food effect investigation. By simply adding two or three targeted administrations using remote controlled capsules to a standard PK study, significantly more information is gathered for a marginal increase

in cost. The term PK*Plus* has been coined to classify this type of study design, exemplified by a recent collaboration with Aventis (Bridgewater, New Jersey),[38] which combined absolute bioavailability and regional drug absorption.

VIII. CONCLUSION

HDA studies performed using specially designed, remote controlled capsules are a convenient, reliable and cost-effective way of gathering fundamental biopharmaceutical data by mapping human intestinal absorption. Through custom study design, it is possible to assess the relative contributions of in vivo dissolution, permeability, gut-wall metabolism, and intestinal efflux on drug development strategy. These data should form an integral part of development pharmaceutics for almost any biopharmaceutically complex compound or delivery system and may therefore underpin key development decisions, such as whether to fast-track the compound, search for a suitable enabling technology, explore other routes of delivery, or perhaps terminate the development.

IX. FUTURE DIRECTIONS

Researchers and regulators continue to search for faster ways to obtain early human ADME data both safely and ethically. Indeed, advances in plasma analytical techniques have made it possible to conduct pharmacokinetic studies with very low drug doses, well below therapeutic levels. This has led to the concept of "human microdosing," where one or perhaps a cocktail of NMEs are administered to assist in lead optimization. In support of this approach, the European Agency for the Evaluation of Medicinal Products (EMEA) has recently adopted a draft position paper outlining the proposed requirements for conducting low-dose clinical screening studies,[39] where the term *low dose* is defined as less than 1/100 of the (systemic) exposure expected to yield a pharmacological effect in humans. Such studies will allow many more compounds to be screened in humans before candidate selection.

By combining microdosing or cassette dosing with a regional absorption study, it is possible not only to obtain human ADME data, but also to gain a very early insight into the biopharmaceutical properties of the compound(s) under study. Studies of this type are likely to prove increasingly popular once the regulatory framework is firmly established.

HDA studies will also advance through developments in commercially available technologies. For example, a likely next generation of the Enterion capsule

will be an extended (or perhaps programmable) release version, so that drug can be infused more slowly or pulsed to different intestinal regions. Future versions could even include an on-board pH sensor, miniaturized video camera, and/or a system for collecting samples of lumenal fluid.

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(54) Drug delivery device which can be retained in the stomach for a controlled period of time.

(57) Drug delivery device retained in the stomach comprising at least one drug and a continuous solid-stick figure, a planar figure or ring figure made from polymer(s) that releases said drug slowly over a controlled, predictable and extended period of time.

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EXHIBIT 2864009224159

IX122Y

TITLE OF THE INVENTION

DRUG DELIVERY DEVICE WHICH CAN BE RETAINED IN THE STOMACH FOR A CONTROLLED PERIOD OF TIME

DESCRIPTION OF THE PRIOR ART

Numerous patents and publications have described devices which can be retained in the stomach for extended periods of time. The prior art can be categorically described according to the technologies listed below:

1.) Floating devices
2.) Swelling devices
3.) Inflating balloons and
4.) For ruminant animals--various shapes.

There follows a discussion of each of the above technologies which demonstrates the technological advancement of the invention over the prior art:

(1) Michaels et al (Alza Corporation), U.S. Patent No. 3,901,232, describe a device which contains a drug delivery compartment attached to an envelope which is contained within an erodible capsule. Upon ingestion, the capsule dissolves and a liquid contained in the envelope vaporizes and swells the envelope to provide a means of retention by flotation within the desired body cavity e.g. the stomach.

S. Watanabe et al, U.S. Patent No. 3,976,764 teach the use of a hollow or low density core surrounded by polymeric materials containing drug(s). The device floats in the stomach and releases said drug over an extended period of time.

Sheth and Tossounian, U.S. Patent Nos. 4,140,755 and 4,167,558 describe the use of a tablet which is hydrodynamically balanced to be buoyant under gastric conditions thereby remaining in the stomach for an extended period of time.

All of the references in this category function on the basis of floatation or buoyancy and do not teach the use of size to retain them in the stomach.

(2) Johnson et al, U.S. Patent No. 3,574,820 teach the use of tablets or capsules containing a reaction product of gelatin and N-acetyl-homocysteine thiolactone as a component of an oral dosage form small enough to be swallowed but which swells in the stomach to become too large to pass through the pylorus.

Banker, U.S. Patent No. 261,242 describes the use of a swellable polymer (e.g. Gantrez) coated on the outside of tablets or capsules such that the coating swells under gastric conditions.

Mamajek and Moyer, U.S. Patent No. 4,207,890 teach the use of an expandable envelope containing a drug and an agent which expands when gastric fluid permeates through the envelope. The device enlarges and is retained in the stomach for an extended period. There is no teaching as to how the device disintegrates or what controls its exit from the stomach.

Theeuwes and Urquhart, U.S. Patent No. 4,434,153 describe the use of a device (containing a hydrogel) which can enter into the gastrointestinal environment where it imbibes fluid and swells 2-50 fold so that it is retained in the stomach over an extended period. Small pills containing drug are released from this device and subsequently delivered for gastric or intestinal absorption.

The patents of this category teach the use of size to retain the device in the stomach and function by absorption of gastric fluid to cause swelling of an expandable polymeric material. No indication of the obtainable duration is given or of control of the process by which the device disintegrates or otherwise is expelled from the stomach.

(3) Alza Corporation, U.S. Patent Nos. 3,797,492 and 3,901,232 disclose the use of inflatable bags containing a gas or vapor-generating system to cause inflation following release of the device from a bioerodible capsule in the stomach (or other

desired body cavity). The inflated envelope causes the device to be retained in the desired site (e.g. stomach) and drug is released from an attached delivery system. When all the drug has been expelled the envelope deflates and the device passes from the stomach.

These patents imply the use of size to retain the device in the stomach and achieve enlarged size in the stomach via conversion of liquid (or solid) components to a gaseous form. They do not teach the use of mechanical movement to create large forms of the device from a smaller configuration.

(4) R. Laby, U.S. Patent No. 3,844,285 describes the use of a device having one initial configuration or adapted to be arranged in the configuration so as to pass into the rumen of an animal, whereupon, it changes to a second configuration which will prevent or hinder regurgitation of the device. This patent is applicable to devices which are delivered to the rumen and required to remain in the rumen by preventing regurgitation. The reference does not teach devices designed to enter and be retained in the stomach of humans or other non-ruminant animals. Neither does it teach any procedure for preventing passage of such devices through the pylorus of humans or non-ruminant animals since only regurgitation is hindered or prevented by the device of the reference. The anatomy of the rumen is quite different from that of the stomach of non-ruminant species.

BACKGROUND OF THE INVENTION

The invention provides a drug delivery device comprising at least one drug and a continuous solid-stick figure, a planar figure or ring figure made from polymer(s) that is retained in the stomach for predictable and extended periods of time for releasing therapeutic or other beneficial agents.

Many orally administered drugs fail to achieve their full potential because of a number of problems including:

a. Slow and incomplete intestinal absorption.

b. Existence of preferential absorption sites in the gastrointestinal tract (absorption windows).

c. Short biological half-life (in particular if the therapeutic index is low).

Solutions to the delivery problems for these agents cannot be guaranteed using conventional controlled release technology since the site of drug release may be beyond the site of optimal absorption or the transit time through the absorbing portion of the gastrointestinal tract may be too short to effect an increase in the duration of action of the drug. In order to optimize the delivery of these agents to achieve maximum effectiveness (and reduce concentration-related side effects) it is desirable to obtain a drug delivery device which would be retained in the stomach for a prolonged, predictable period of time during which it would release the agent in a predetermined pattern. At the end of its period of usefulness, the device would disintegrate or otherwise alter its properties such that it would exit from the stomach and pass down the intestine.

Accordingly, it is an object of the invention to provide a means of retaining the dosage form in the stomach for an extended, predictable period of time. Thereby the duration of absorption and hence the duration of action of drugs with short biological half-lives could be extended. Likewise the bioavailability of the agents could be improved over that achieved from a conventional dosage form or conventional sustained release preparation.

Another object of the invention is to provide a mechanism whereby after a predetermined time the device will erode, disintegrate or otherwise alter its properties and pass out of the stomach and into the intestine. In addition the device should not lodge in the intestine thereby causing an obstruction.

A further object is to devise a device which will not obstruct the passage of food while the device is in the stomach or after it has passed into the intestine.

Other objects, features and advantages of the invention will be apparent to one skilled in the art from the detailed description of the invention which follows.

Immediately after consumption of food, the stomach acts as a holding tank for the solids while they are digested and broken down into small particles (1-2 mm in diameter) by the action of acid, enzymes and the physical grinding of the food particles by muscular contractions of the stomach wall. Due to the sieving action of the pyloric valve only particles of the order of 1-2 mm leave the

stomach and pass into the intestine during the digestive period. Approximately two hours after digestion of a meal, the stomach passes into its interdigestive phase during which there are regular and frequent (every 1-1/2 - 2 hours) periods of intense contraction known as the interdigestive migratory myoelectric complex (IMMC). These contractions which can apply forces up to 200 cm of water (approximately 0.2 atmospheres pressure) are designed to remove any remaining ingested food from the stomach through a fully open pylorus. Clearly, then, any dosage form which is designed to remain in the stomach for an extended period (i.e., greater than a few hours) must be capable of withstanding these contractive forces to prevent it from passing from the stomach into the intestine.

A BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a preferred configuration of a continuous solid-stick figure device in a tetrahedral form with fastened ends.

Figure 1a is a preferred configuration of the device in a tetrahedral form.

Figure 1b is formed when corner 1 and 3 and corners 2 and 4 of Figure 1a are compressed together.

Figure 1c refers to encapsulation of Figure 1(b).

Figure 2 is a preferred configuration of a planar figure device in a 4-lobed planar form.

Figures 2a and 2b show the device of figure 2 folded and inserted into a capsule for oral administration.

Figure 3 is a preferred configuration of the device in a planar, disc-shaped form.

Figures 4a and 4b show the device of figure 3 folded and inserted into a capsule for oral administration.

Figure 5 is a preferred configuration of the device in a planar, 4-limbed cross form. Each limb of the device contains a rigid, soluble polymer which slowly erodes resulting in loss of integrity of the device.

Figures 6a and 6b show the device of figure 5 folded and inserted into a capsule for oral administration.

Figure 7 is a preferred configuration of a ring figure device in a ring form.

Figure 8a shows the device in a folded configuration.

Figure 8b refers to encapsulation of Figure 8a.

DESCRIPTION OF THE INVENTION

The invention is directed to a gastric retention device comprising a continuous stick figure prepared from at least one erodible polymer, said device having the following properties:

a) compressible to a size suitable for swallowing;

b) expandable to size which will prevent passage through the pylorus for a predetermined time;

c) sufficiently resistant to a simultaneous force in two directions by

a stomach to prevent passage through a pylorus for a predetermined time; and

d) erodible in the presence of gastric juices so that said device after a predetermined time is no longer able to retain or attain the expanded configuration defined in (b) above and/or resist a simultaneous force in two directions as defined in (c) above.

The invention is also directed to a method for the controlled release over a period of time of a drug in the stomach, comprising a continuous stick figure prepared from at least one erodible polymer, said device having the following properties:

a) compressible to a size suitable for swallowing;

b) expandable to size which will prevent passage through a pylorus for a predetermined time;

c) sufficiently resistable to a simultaneous force in two directions by a stomach to prevent passage through a pylorus for a predetermined time; and

d) erodible in the presence of gastric juices so that said device after a predetermined time is no longer able to retain or attain the expanded configuration defined in (b) above and/or resist a simultaneous force in two directions as defined in (c) above.

Definitions:

Gastric retention device is a device which resides in the confines of the stomach for the purpose of providing a platform for controlled release of biologically active agents.

Continuous solid-stick figure refers to a framework composed of more or less rigid rods or sticks (preferably a Tensile Modulus of at least 1 X 10^3 to 50 X 10^6 psi, more preferably in the range of 1.5 X 10^4 to 20 X 10^6 psi) which are bent and/or fastened together in a manner such that the framework will not pass out of the stomach, but will allow passage of food. Preferred configurations are illustrated in Figures 1, 1a, 1(b) and 1(c).

The expanded tetrahedral device shown in Figures 1 is compressed by squeezing together corners 1 and 3 and corners 2 and 4 of Figure 1(a) by applying forces at right angles to subsequently obtain the form of Figure 1(b) and ultimately inserted into a hard gelatin capsule as illustrated in Figure 1(c).

A planar disc-shaped or multi-lobed flat or planar device may be used which is large enough and rigid enough such that said device will not pass out of the stomach but will allow passage of food around said device. Preferred configurations are illustrated in Figures 2-6.

The longest diamerter (the device need not be symmetrical) of the device may vary between 1.6 and 5 cm; more preferably in the range 2.0 to 4 cm; and most preferably in the range 3.2 to 3.6 cm.

The expanded devices shown in Figures 2, 3 and 5 are compressed by folding or rolling to

subsequently obtain the form of Figures 2a, 4a, and 6a respectively and ultimately inserted into capsules as illustrated in Figures 2b, 4b and 6b.

A ring refers to an annulus as shown in Figure 7. The ring is preferably made of a non-erodible extruded rod-shaped material and then merely joining the ends with an erodible material. However, the whole ring may be made of erodible material.

The diamerter of the ring may vary between 1.6 and 5 cm; more preferably in the range 2.5 to 4 cm; and most preferably in the range 3.2 to 3.6 cm.

The cross-section of the band making up the circumference of the annulus may be one of many shapes (e.g.., circular, rectangular, square, triangular and the like). The dimensions of the cross-section should be in the range 0.5 to 5 mm, more preferably in the range 1 to 3 mm. The cross-section may be solid or hollow; i.e., the ring may be constructed from a tube which may be empty or may contain liquid, solid or gaseous material.

The ring shown in Figure 7 may be folded as shown in Figure 8a and inserted into a capsule (Figure 8b) for oral amdinistration.

Criterion A means that the desired expanded configuration of said device is too large to be swallowed and thus must be compressed and contained in a conventional capsule or like container for swallowing. The capsule is designed to dissolve after oral ingestion within the confines of the stomach.

Criterion B means that after the compressed device within the capsule container reaches the stomach, upon dissolution and/or disintegration of the retaining capsule or like container, said device

will expand or unfold in such a manner that results in its original shape which is too large to pass from the stomach into the intestine unless enough force is applied to recompress the device. This "expanded" form of the device will remain in the stomach for a pre-determined period of time depending on the desired time profile of release of the biologically active agent, which is a part of the device or contained within a controlled release module that is attached to the retention device. The "pre-determined time" is preferably within the range of 1 hour to 1 year. For example, a biologically active agent which prevents heart-worm infestation in dogs might require gastric retention and controlled delivery of said agent over the course of 6-12 months. This period depends on the seasonal changes of the mosquito population which is the main vector of heart worm infection in dogs. A second example is the use of such a gastric retention device to maintain controlled delivery of a contraceptive agent. The desired retention time of the device might be 3-4 weeks to coincide with the normal frequency of menses. A third example is the use of such a device to achieve once daily dosing, if such device is coupled to a controlled release device for delivery of an agent with a very short half-life of biological activity, such as a dopamine agonist for treatment of Parkinsonism.

Criterion C means that the device in question must sustain some compressive force, in excess of that amount the stomach is able to apply, in order for said device to not prematurely pass on into the

intestine. For humans and animals with similar gastric anatomy such as dogs, the gastric pouch is capable of applying forces of 50-150 grams.

Criterion D means that after some pre-determined time, during which said device has been utilized, some part of the device will suddenly or gradually fail to meet Criteria B and/or C. That is, the device will either be unable to withstand a lesser compressive force, or will lose its integrity as a single unit, and thereby be subject to the normal propulsive forces and pass out of the stomach. By doing so, the danger of multiple devices causing obstructive problems, is obviated.

These devices can be manufactured by a number of processes including injection molding, extrusion, film-forming, laminating and the like as will be clear to one skilled in the art. For example, a mold can be constructed in the desired shape of the device and filled with appropriate material(s) in the liquid state and then allowed to cure by chemical processes or cooling if thermosetting materials(s) is used.

A critical property of the devices is that, after a certain, pre-determined period of time in the stomach they will alter their properties in a manner which will permit their elimination from the stomach into the intestine. For this purpose the devices must contain components(s), sections(s) or points(s) which will erode or dissolve in the aqueous environment of the stomach.

For example, the four lobes of the device of figure 2 may be fastened at a central point by glue (or other material) which will dissolve or erode

during the desired time period, thereby liberating the individual lobes which will then easily pass out of the stomach.

The disc can be manufactured from composite mateirals such that one or more components(s) will erode or dissolve leaving a disc whose mechanical properties are such that it can be readily removed from the stomach by the compression forces associated with the recurrent interdigestive migratory myoelectric complex.

A further modification of the four-lobed form is to have contained within the lobes an erodible or dissolving structure which will maintain the lobes in a rigid configuration until the internal, structurally supporting material has disintergrated. At that point the device would be incapable of maintaining its rigid, planar conformation and would be eliminated from the stomach. (see figure 3 and Example 11).

Although the polymers may be blends, combinations, composites or copolymers (erodible and non-erodible), said polymers must be erodible at the point(s) of desired dissolution and/or disintegration. Representative erodible polymers which can be employed in the practice of the invention are cellulosics such as Klucel (hydroxypropylcellulose), cellulose acetate phthalate, methyl cellulose, hydroxypropylmethyl-cellulose phthalate and the like; ethylene/vinyl alcohol copolymer; ethylenemaleic anhydride copolymer; polyacrylates such as Eudragit E (cationic copolymer based on dimethylaminoethyl methylacrylate and neutral methylacrylic acid esters), poly(acrylic acid), poly(methacrylic acid) and the like; polylactones such as poly(caprolactone) and the like;

polyanhydrides such as poly[bis-(p-carboxyphenoxy)-propane anhydride], poly(terephthalic acid anhydride) and the like; polyvinyl pyrrolidone; polyamides and polypeptides such as polylysine, polyglutamic acid and the like; gelatin and derivatives such as those produced on reaction with N-acetyl-homo-cysteine thiolactone and the like; polyesters such as polylactides, polyglycolides, poly(betahydroxybutyric acid) and the like; poly(ortho esters) such as copolymers of DETOSU with diols such as hexane diol, decane diol, cyclohexane dimethanol, ethylene glycol, polyethylene glycol and incorporated herein by reference those poly(ortho) esters described and disclosed in U.S. Patent No. 4,304,767 and the like; polyurethanes such as those prepared with poly(tetramethylene oxide), prepolymer terminated with hexamethylene diisocyanate and a poly functional diester of oxalic acid and glycerol, di-(2,3-dihydroxypropyl)oxalate or its mixture with 3,6-dioxaoctane-1,8-diol and the like; polyacrylonitriles such as poly(alkyl-α-cyano-acrylates) wherein the alkyl is methyl, ethyl, propyl, butyl, amyl and the like; and types of inorganic glass based on polyphosphates and fused salts.

Representative non-erodible polymers that may be employed in the practice of the invention are polyolefins such as polyethylene, polypropylene, ethylene vinyl acetate copolymers, poly(tetrafluoro-ethylene) and the like; rubbers such as silicon based rubber, styrene-butadiene copolymers and the like polyamides such as nylon 6,6, nylon 6, and the like; polyesters such as poly(ethylene terephthalate) and

the like; polyurethanes formed from diisocyanates such as 1,6-hexane diisocyanate or biphenylene diisocyanate etc. and diols such as ethylene glycol, 1,4-butane diol and the like; and cellulosics such as ethyl cellulose, cellulose diacetate, cellulose triacetate and the like.

A drug or medicament may be associated with the gastric retention device in different ways, depending on the physical and chemical properties of the drug or medicament. For example, the drug may be dispersed as a solution or suspension within an erodible polymer matrix such that as the matrix erodes within the confines of the gastric pouch, the drug is released at a predetermined rate. Similarly, the drug may be dissolved or dispersed within a non-erodible matrix material comprising part of the retention device. As this matrix comes in contact with gastric fluid, the drug diffuses out of the non-erodible matrix at a predetermined rate. An alternative to incorporating the drug as an integral part of the gastric retention device is to simply fasten a controlled release drug module to the retention device. Such a module might be a miniature constant-flow pump, either mechanically or osmotically driven, and may be fastened to the retention device by gluing or tethering. Likewise it may consist of a matrix system, erodible or non-erodible, fastened to the retention device.

In man and similarly-sized non-ruminate mammals, the size of the pyloric valve between the stomach and small intestine is generally in the range of 3-5 cm maximum inner circumference. Objects with a mimimum circumference of more than 5 cm will

generally not pass from the stomach through the pylorus. The stick-figure, planar figure and ring figure devices described herein are designed to present a minimum circumference of more than 5 cm after deployment in the stomach. Before deployme in the stomach the minimum circumference is less than 2 cm.

To further set forth the concept of the invention several gastric retention studies are shown below.

Studies were performed with Beagle dogs in order to ascertain gastric retention time of the drug delivery device described herein. The parameters investigated were size, erodibility and polymer flexibility. These devices were administered in gelatin capsules and their position in the gastro-intestinal tract determined using x-ray techniques. Each device was tested in three to four different dogs in three fed states: fed (fed 10 minutes before dosing and food ad lib thereafter) fed/fast (fed 10 minutes before dosing, and fasted thereafter for 36 hours), and fast (fasted 18 hours before dosing and thereafter for 36 hours).

The results for a tetrahedral device of different dimensions are shown below.

Shape	Dimensions	Average % Retained 24 hours	N*
Tetrahedron	2.0 cm (wide) x 2.0 cm (long) x 2.0 cm (high)	92	12
	1.5 cm x 1.5 cm x 1.5 cm	85	12

*Number of trials.

Polymer flexibility studies were also carried out in Beagle dogs. One point five millimeter (1.5 mm) diameter rods having varying flexibilities were extruded. These rods were constructed of polyethylene alone, polyethylene blended in various proportions with a copolymer of ethylene (86%) and vinyl acetate (16%) or the copolymer of ethylene and vinyl acetate alone. All rods also contained 15% by weight of barium sulfate to render them visible by x-ray in the g.i. tract of the dogs.

Shape	Material	Tensile Modulus (psi)	% Retained at 24 hours	N
	100% PE	11,556	92	12
Tetrahedron	PE 94%/EVA 6%	11,383	57	7
(2 cm)	PE 30%/EVA 70%	9,512	50	4
	100% EVA	3,828	50	4

EVA composition 86% ethylene, 14% vinylacetate
PE is polyethylene

Studies were conducted on the degree of erodibility of the polymeric materials described within the invention. A series of poly(ortho ester)/polyethylene (POE/PE) blends have been tested in Beagle dogs using a modified tetrahedron device. The tetrahedron shape was formed from four silastic, 15% barium-load corners with openings for insertion of the poly(ortho ester)s/polyethylene rods. The corner pieces were 3 mm thick and formed an equilateral triangle. The completed tetrahedron measured 2x2x2 cm (see figure I). All dogs were tested in the fasted state (fasted 18 hours before dosing and thereafter for 36 hours) and the following data obtained.

**POE/PE	% Retained at 24 hours	N
50/50	80	5
65/35	80	5
75/25	100	5
80/20	0	4
85/15	25	4
90/10	0	5

**POE used was prepared from cis-trans cyclohexanedimethanol and 3,9-Bis(ethylidenyl)-2,4,8,10-tetraoxaspiro[5,5]undecane [Detosu]

In vitro dissolution studies were carried out with the poly(ortho ester)/polyethylene blends. One inch pieces of the 1.5 mm diameter rod blends were tested in pH 1.5 buffer, in a 37°C water/shaker bath. At selected time intervals a piece was removed

from the water/shaker bath and measured gravimetrically. Percent erosion was calculated from weight loss as shown below.

In Vitro Dissolution - % Poly(ortho ester) Eroded

	Poly(ortho ester)/Polyethylene				
Time (hours)	50/50	65/35	75/25	80/20	85/15
8	35.4	40.9	53.2	95.1	99.3
16	55.2	77.2	86.9	97.9	97.3
24	72.0	84.0	84.4	98.8	96.6
28	74.6	90.5	86.4	98.1	96.8
32	87.4	88.2	87.5	98.3	98.4

The experimental error is about ±10%.

Studies were performed with Beagle dogs in order to ascertain gastric retention time of the drug delivery device described herein. The parameters investigated were size, erodibility and polymer flexibility. These devices were administered in gelatin capsules and their position in the gastrointestinal tract determined using x-ray techniques. Devices were tested in three to four differing dogs which were fasted 18 hours before dosing and thereafter for 36 hours. The effect of size (ring diameter) on gastric retention time is shown in Table I below.

TABLE I

Shape	Dimenions	Average % Retention at 24 Hours	N*
Ring	3.6 cm diameter	100	6
Ring	2.5 cm diameter	70	10

*N=Number of trials

Polymer flexibility studies were also carried out in Beagle dogs. One point five millimeter (1.5 mm) diameter rods having varying flexibilities were extruded. These rods were constructed of polyethylene alone, polyethylene blended in various proportions with a copolymer of ethylene (86%) and vinyl acetate (16%) or the copolymer of ethylene and vinyl acetate alone. These rods were formed into rings and inserted into capsules for *in vivo* testing. Rings were also constructed from Silastic™ (Dow Company) medical grade silicone rubber. All rings also contained 15% by weight of barium sulfate to render them visible by x-ray in the g.i. tract of the dogs. See Table II below.

TABLE II

Shape	Material	Tensile Modulus (psi)	% Retained at 24 hours	N
	100% PE	11,556	100	6
Rigid Ring	PE 94%/EVA 6%	11,383	100	4
(3.6 cm)	PE 30%/EVA 70%	9,512	75	4
	100% EVA	3,828	0	4
Soft Ring	silicone rubber	ND*	67	9
(3.0 cm)				

EVA composition 86% ethylene, 14% vinylacetate
PE is polyethylene
*N.D.=not determined. This ring was very flexible.

To demonstrate that a ring could rapidly exit from the stomach when its integrity was lost, unsealed rings (i.e. strings) were administered to dogs in gelatin capsules and then gastric retention measured by X-ray techniques. The data are shown in Table III below:

III

Shape	Material	Size	Gastric Retention Time (hours)	N
String	Silastic	6 cm long 1.5 mm diameter	40-70	2

These results demonstrate that if a ring contains an erodible section which can be ruptured at a predetermined time, the resultant string will be eliminated from the stomach.

The erodible section(s) can be constructed from many different types of material. Examples of erosion profiles of some such materials (blends of polyethylene and a poly(orthoester) are shown in the Table IV below.

EXHIBIT 2

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In Vitro Dissolution - % Poly(ortho ester) Eroded

Time (hours)	Poly(ortho ester*)/Polyethylene 50/50	65/35	75/25	80/20	85/15
					(% by weight)
8	35.4	40.9	53.2	95.1	99.3
16	55.2	77.2	86.9	97.9	97.3
24	72.0	84.0	84.4	98.8	96.6
28	74.6	90.5	86.4	98.1	96.8
32	87.4	88.2	87.5	98.3	98.4

The experimental error is about ±10%.

*The poly(orthoester) used was a copolymer of cis-trans cyclohexane dimethanol and 3,9-Bis-(ethylidenyl)-2,4,8,10-tetraoxaspiro[5,5]undecane (Detosin).

The active agents (therapeutic agent or other beneficial agents) which can be utilized in accordance with the practice of the invention is not critical. Any agent which can be obtained in a stable form is applicable herein. As to the amount of active agent which can be delivered in accordance with the invention, said amount depends on a variety of factors such as the host, physical attributes, particular disease or disorder being treated and of course the severity of the condition being treated. The amount of active agent utilizable in the invention is known to one skilled in the art and is

disclosed in various medical references such as PDR and etc. or could be obtained from suitably designed clinical trials.

Generally, the amount of polymer employed in the practice of the invention ranges from 10% to 99.9% polymer (preferably 20% to 60%) by weight of the drug delivery platform. The remaining portion of the composition contains the medicament and conventional pharmaceutically acceptable excipients.

Representative pharmaceutically acceptable excipients that the drug delivery device may contain are buffering agents and preservatives. Suitable water soluble preservatives which may be employed in the drug delivery device are sodium bisulfite, sodium thiosulfate, ascorbate, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric borate, parabens, benzyl alcohol and phenylethanol. These agents may be present in amounts from 0 to 5% by weight. Suitable water soluble buffering agents are alkali or alkali earth carbonates, phosphates, bicarbonates, citrates, borates, acetates, acid anhydrides, succinates and the like, such as sodium phosphate, citrate, borate, acetate, bicarbonate and carbonate. These agents may be present in amounts sufficient to maintain some optimum pH of the system in the range 1 to 9. As such the buffering agent can be as much as 25% on a weight to weight basis of the total composition.

The following examples illustrate the preparation of various drug delivery devices of the invention. The Examples should be construed as illustrations rather than limitations thereof.

EXAMPLE 1

A modified tetrahedron was constructed from four Silastic (polydimethylsiloxane) 15% barium-loaded corners with openings for insertion of the poly(ortho ester) (65/35 HD/tCDM)/polyethylene blend rod (Fig. 1). The completed tetrahedron measured 2x2x2 cm and fit into a 000 gelatin capsule. A biologically active compound may be incorporated into the erodible rod or the silastic corners.

The poly(ortho ester) (a copolymer of 65% hexane diol, 35% trans-cyclohexane dimethanol with DETOSU 65/35 HD/tCDM)/polyethylene blends were tested in Beagle dogs using x-ray techniques. All dogs were dosed in the fasted state (fasted 18 hours before dosing and thereafter for 36 hours). (See Table IV).

Table IV

Material	Percent Retained at 24 hrs in Stomach	N*
Poly(ortho ester) (65/35 HD/tCDM)/polyethylene		
50/50 Blend	100	4
60/40 Blend	67	3

*Number of trials.

EXAMPLE 2

Following the procedure of Example 1, the rods that formed the tetrahedral configuration of the stick figure were fabricated from Klucel HF

(hydroxypropylcellulose)/polyethylene blends. In vivo performance in dogs is shown in Table V.

Table V

Material	Percent Retained at 24 hrs in Stomach	N
Klucel HF/polyethylene		
75/25 Blend	100	4
90/10 Blend	50	2

EXAMPLE 3

Following the procedure of Example 1, the rods that formed the tetrahedral configuration of the stick figure were fabricated from poly(ortho ester) (c,tCDM)/polyethylene blends. Results are given in the following tables. In vivo performance in dogs is shown in Table VI.

Table VI

Poly(ortho ester) (c,tCDM***)/Polyethylene	Percent Retained at 24 hrs in Stomach	N
50/50	80	5
65/35	80	5
75/25	100	5
80/20	0	4
85/15	25	4
90/10	0	5

*** c,tCDM represents a copolymer of cis,trans cyclohexanedimethanol with Detosu.

EXAMPLE 4

Following the procedure of Example 1, the rods that formed the tetrahedral configuration of the stick figure may be fabricated from blends of poly(ortho ester) (65/35 HD/tCDM)/polypropylene.

EXAMPLE 5

Following the procedure of Example 1, the rods that formed the tetrahedral configuration of the stick figure may be fabricated from blends of Hydroxypropylmethylcellulose phthalate (HPMCP)/ polyethylene.

EXAMPLE 6

Following the procedure of Example 1, the rods that formed the tetrahedral configuration of the stick figure may be fabricated from Eudragit E with methylcellulose.

EXAMPLE 7

This example demonstrates the utility of a tetrahedral configuration that may be made solely from blends of poly(ortho ester) (65/35 HD/tCDM)/ polyethylene. The completed tetrahedron measures 2x2x2 cm and fits into a number "0" gelatin capsule for dosing (Fig. 1a, b and c). A biologically active compound may be incorporated into the erodible material or otherwise contained in an attached drug reservoir via conventional formulation procedures.

EXAMPLE 8

Following the procedure of Example 7, the material that formed the tetrahedral shape may be fabricated from a poly(ortho ester) (65/35 HD/tCDM)/ EVA blend.

EXAMPLE 9

Following the procedure of Example 7, the material that formed the tetrahedral shape may be fabricated from a Klucel HF/polypropylene blend.

EXAMPLE 10

Studies were performed with Beagle dogs in order to ascertain gastric retention time of the drug delivery device described herein. The parameters investigated were size, shape and erodibility. These devices were administered in gelatin capsules and their position in the gastrointestinal tract determined using x-ray techniques.

The results for disc and 4-lobed planar devices of different dimensions and made from Silastic (Dow Company) medical grade silicone rubber labelled with 15% barium sulfate are shown below.

Shape	Dimensions	% Retained at 24 hrs.	N*
Disc	2.5 cm diameter	67	9
4-lobed figure	3.2 cm diameter	90	10
	2.7 cm diameter	83	12
	2.2 cm diameter	60	10

* Number of trials

These data indicate that the retention of these devices in the stomach is a function of their diameter and that retention for a period of 24 hours is possible using devices which can be folded into gelatin capsules suitable for swallowing.

EXAMPLE 11

The effect of erosion on the performance of one of the devices is demonstrated in this example. The planar cross form shown in figure 5 was constructed containing segments of rigid, bioacceptable material. For this example segments of spaghetti were inserted into hollows in the limbs of the cross. When the device came into contact with water, the spaghetti segments absorbed water and lost their rigidity. The overall effect was to produce a device which had lost its integrity and which could be more readily expelled from the stomach by the forces of the interdigestive migratory myoelectric complex. The data from one such experiment using Beagle dogs are shown below.

Shape	% Retained in stomach at 24 hrs.	N
cross with non-eroding limbs	100%	2
cross with eroding limbs (spaghetti)	71%	7
cross with empty limbs	57%	7

The data show that the cross-form with hollow limbs is much less-well retained than the same shape with rigid, non-eroding limbs. The device with initially rigid, but eroding, limbs was intermediate in performance.

EXAMPLE 12

The ring may be fabricated entirely from one material which is erodible or water soluble so that it slowly disintegrates in the confines of the stomach and either breaks up into smaller pieces which are expelled or is no longer able to withstand the contracting forces of the stomach wall and thereby violates criterion (c).

EXAMPLE 13

The ring can be formed from a blend or composite of two or more materials at least one of which is water soluble or erodible which results in predetermined expulsion of the device from the stomach as in Example 12 above.

EXAMPLE 14

Example 12 or 13 where the erodible material is a polyorthoester.

EXAMPLE 15

Example 12 or 13 where the water soluble material is hydroyxpropylcellulose.

EXAMPLE 16

The ring can be constructed from a linear portion of erodible or non-erodible material whose ends are joined to form a ring using an erodible or water soluble material. When this joint disintegrates the device will resort to the form of a linear rod or string which can then be expelled from the stomach.

EXAMPLE 17

Example 16 where the erodible joint is made of a poly(orthoester).

EXAMPLE 7

Example 16 where the water soluble joint is made of hydroxypropylcellulose.

EXAMPLE 8

Example 12 where the drug is contained in the erodible matrix of the ring.

EXAMPLE 9

Example 12 where the drug is contained in a module which is attached to the ring.

WHAT IS CLAIMED IS:

1. A gastric retention device comprising a continuous solid-stick figure, a planar figure or ring figure prepared from at least one erodible polymer, said device having the following properties:

a) compressible to a size suitable for swallowing;

b) expandable to size which will prevent passage through a pylorus for a predetermined time;

c) sufficiently resistant to a simultaneous force in two directions by a stomach to prevent passage through a pylorus for a predetermined time; and

d) erodible in the presence of gastric juices so that said device after a predetermined time is no longer able to retain or attain the expanded configuration defined in (b) above and/or resist a simultaneous force in two directions as defined in (c) above.

2. The device of Claim 1 wherein said erodible polymer is selected from the group consisting of soluble cellulosic materials, ethylene vinylalcohol, ethylenemaleic anhydride copolymer, polyacrylates, polycaprolactones, inorganic glass based on polyphosphates and fused salts, polyanhydrides, poly(ortho)esters, biodegradable polyurethanes, polyvinyl pyrrolidone, polylactones, polyamides and polypeptides, gelatin and derivatives, polyacrylonitriles, polyesters and combinations thereof.

3. The device of Claim 1, wherein said device is prepared from at least one erodible polymer and at least one non-erodible polymers selected from the group consisting of polyolefins, ethylenevinylacetate copolymers, rubbers, ethylene-vinylalcohol copolymers, polyamides, polyurethanes, polyesters, teflon, non-water-soluble cellulosic and combinations thereof.

4. The device of Claim 1 in stick figure form, wherein said figure is from 1 to 3 cm along each leg of said figure; wherein said erodible polymer is selected from the group consisting of polyolefins, ethylenevinylacetate copolymer, ethylenevinylalcohol copolymer, poly(ortho)esters, cellulosics, polyanhydrides, polyamides and polypeptides, polyphosphates and fused salt and combinations, composites, blends and copolymers thereof; wherein the arms of said device have a Tensile Modulus of at least 1×10^3 to 50×10^6 psi; and wherein said predetermined time is from one (1) hour to one (1) year.

5. The device of Claim 4, wherein said polyolefin is polyethylene or polypropylene; and said cellulosic is hydroxypropylcellulose, cellulose acetate phthalate or ethyl cellulose.

6. The device of Claim 4, wherein said stick figure is from 1.5 to 2.5 cm along each leg of said figure; said polymer is selected from the group consisting of a mixture of poly(ortho) ester/

polyethylene, a mixture of ethylene/vinylacetate, poly(ortho) ester and polyethylene; and said predetermined time is from 16 to 48 hours.

7. The device of Claim 6, wherein said polymer is a mixture of poly(ortho ester) and polyethylene.

8. The device of Claim 7, wherein said mixture is 75/25 poly(ortho ester)/polyethylene.

9. The device of Claim 1 wherein said polymer is a poly(ortho) ester.

10. The device of Claim 1 in ring figure form, wherein said ring figure is from 1.6 to 5 cm in diameter, wherein said erodible material is selected from the group consisting of polyolefins, ethylenevinylacetate copolymer, ethylenevinylalcohol copolymer, poly(ortho)esters, cellulosics, polyanhydrides, polyamides and polypeptides, polyphosphates and fused salt and combinations, blends, composites and copolymers thereof; and wherein said predetermined time is from one (1) hour to one (1) year.

11. The device of Claim 10, wherein said polyolefin is polyethylene or polypropylene; and said cellulosic is hydroxypropylcellulose, cellulose acetate phthalate or ethyl cellulose.

12. The device of Claim 10, wherein said ring figure is from 3.2 to 3.6 cm in diameter, said material is selected from the group consisting of a

mixture of poly(ortho) ester/polyethylene, a mixture of ethylene/vinylacetate, poly(ortho) ester and polyethylene; and said predetermined time is from 16 to 48 hours.

13. The device of Claim 12, wherein said material is a mixture of poly(ortho ester) and polyethylene.

14. The device of Claim 13, wherein said mixture is a 75/25 weight ratio of poly(ortho ester)/polyethylene.

15. The device of Claim 1 in planar figure form, wherein said planar figure is from 1.6 to 5 cm in diameter; wherein said erodible polymer is selected from the group consisting of polyolefins, ethylenevinylacetate copolymer, ethylenevinylalcohol copolymer, poly(ortho)esters, cellulosics, polyanhydrides, polyamides and polypeptides, polyphosphates and fused salt and combinations, blends and copolymers thereof; and wherein said predetermined time is from one (1) hour to one (1) year.

16. The device of Claim 15, wherein said polyolefin is polyethylene or polypropylene; and said cellulosic is hydroxypropylcellulose, cellulose acetate phthalate or ethyl cellulose.

17. The device of Claim 16, wherein said planar figure is from 2 to 4 cm in diameter said polymer is selected from the group consisting of a

mixture of poly(ortho) ester/polyethylene, a mixture of ethylene/vinylacetate, poly(ortho) ester and polyethylene; and said predetermined time is from 16 to 48 hours.

18. The device of Claim 17, wherein said polymer is a mixture of poly(ortho ester) and polyethylene.

19. The device of Claim 18, wherein said mixture is 75/25 poly(ortho ester)/polyethylene.

EXHIBIT 2

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1/6

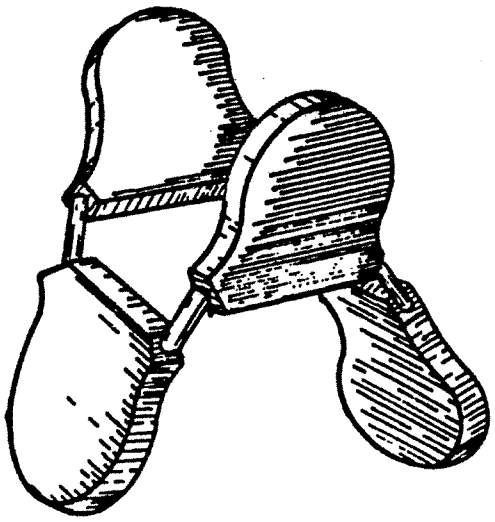

Fig. 1

EXHIBIT 2

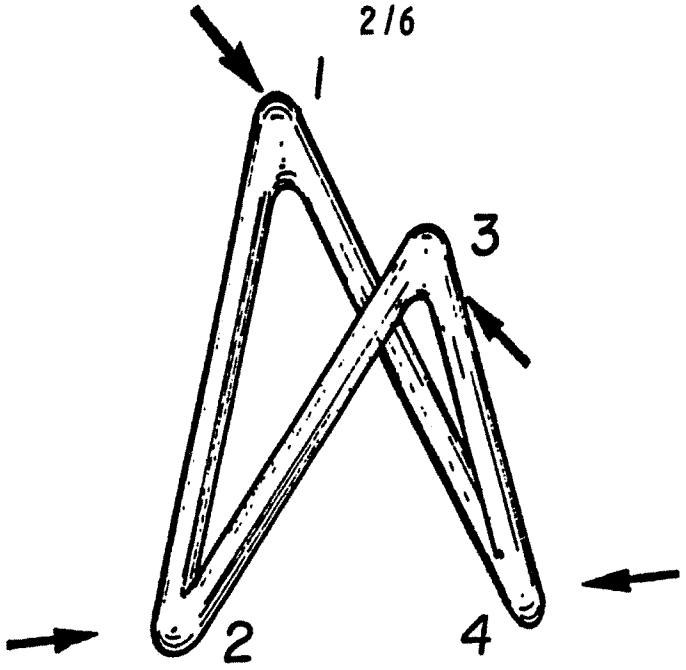

Fig. 1(a)

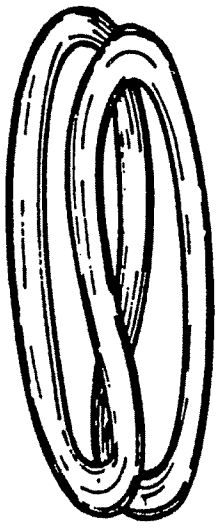

Fig. 1 (b)

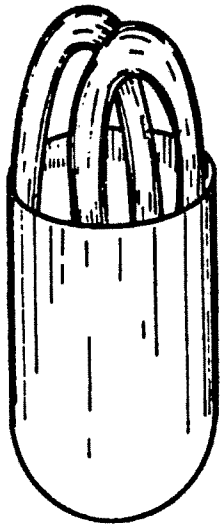

Fig. 1 (c)

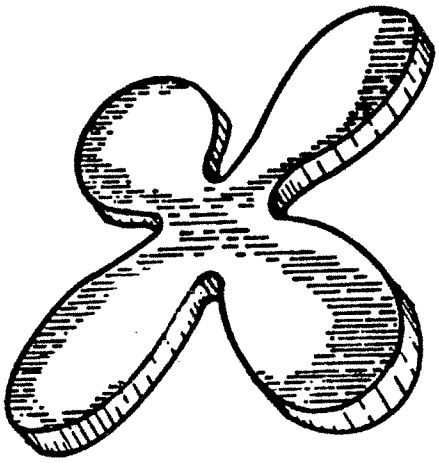

Fig. 2

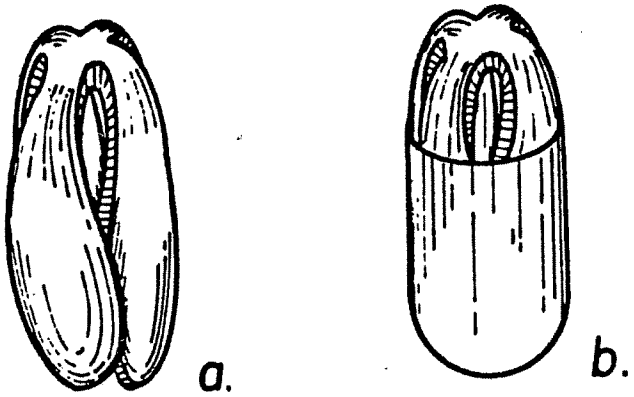

Fig. 2

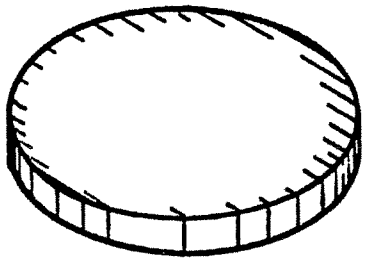

Fig. 3

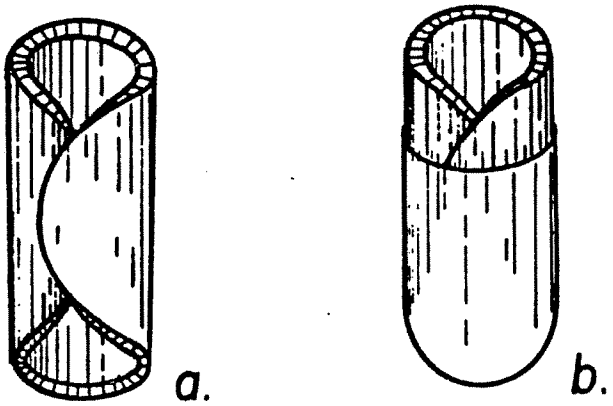

Fig. 4

EXHIBIT 2

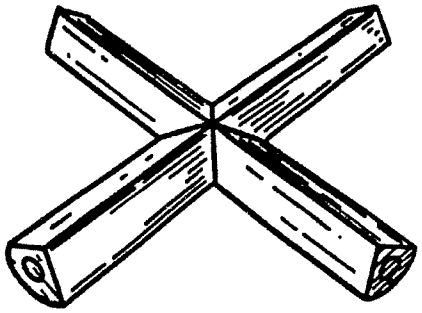

Fig. 5

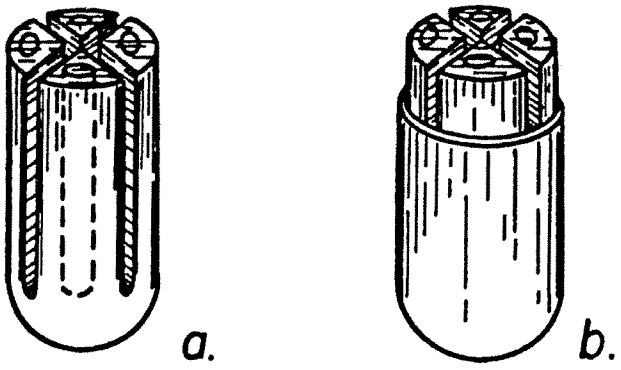

Fig. 6

EXHIBIT 2

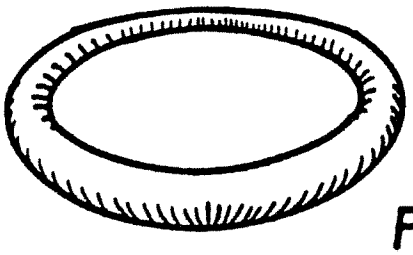

Fig. 7

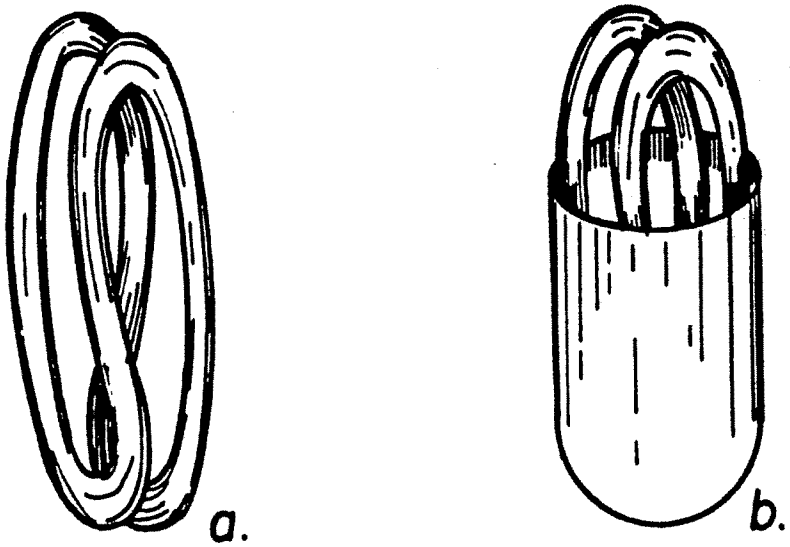

Fig. 8

EXHIBIT 3

University of Utah Hospitals & Clinics

New Drug Bulletin: Methylphenidate Transdermal System (Daytrana™ - Shire Pharmaceuticals)

October 12, 2006

Written by LeeAnn Miles, PharmD Student
Edited by Erin Fox, PharmD, Drug Information Specialist

Methylphenidate transdermal system (Daytrana™) received FDA approval on April 4, 2006 and is the first methylphenidate patch for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) in children ages 6-12 years old. Methylphenidate is a central nervous system (CNS) stimulant thought to inhibit the reuptake of norepinephrine and dopamine presynaptically, and enhance the release of catecholamines into the synaptic cleft.

Serum concentrations of d-methylphenidate after a single application of the patch are similar to those achieved with a single dose of once-daily oral methylphenidate formulations. Time to peak serum concentrations ranges from 7.1-8.8 hours. After multiple applications, serum concentrations are up to 1.9 times higher than those found with oral formulations, indicating that absorption of methylphenidate increases with continued use. Methylphenidate is highly lipophilic and 10-33% protein-bound, giving it the ability to efficiently penetrate the CNS. Methylphenidate is metabolized by de-esterification to the inactive metabolite a-phenyl-piperidine acetic acid (ritalinic acid), which is excreted in the urine. The mean elimination half-life is 3-4 hours.

Two randomized double-blind, placebo-controlled trials demonstrated the efficacy of the Daytrana™ in children aged 6 to 12 years old. Mean changes from baseline in both the SKAMP-D scale and the ADHD-Rating Scale-IV showed statistically significant improvements in children receiving Daytrana™ compared to placebo. There are no data for the use of Daytrana™ in the treatment of adults with ADHD.

The most common adverse effects associated with Daytrana™ are decreased appetite (26%), insomnia (13%), nausea and vomiting (10-12%), weight loss (9%), tic (7%), affect lability (6%), and nasal congestion or nasopharyngitis (5-6%). Early removal minimizes late day side effects such as insomnia or decreased appetite. Application site irritation (erythema) and contact sensitization (intense local reaction with edema, papules, or vesicles) are possible with the use of the patch, but incidence is low when applied for 9 hours per day, alternating right and left hip areas.

Methylphenidate inhibits the metabolism of warfarin, phenytoin, phenobarbital, primidone, tricyclic antidepressants, and selective serotonin reuptake inhibitors. Patients taking these medications concurrently may need dose adjustments. Because methylphenidate can increase blood pressure, it may decrease the efficacy of antihypertensive medications. Methylphenidate is contraindicated with monoamine oxidase inhibitor therapy. It is also contraindicated with agitation, glaucoma, tics or family history of Tourette's syndrome, and hypersensitivity to methylphenidate.

Apply the patch to the hip area once daily in the morning 2 hours prior to needed effect and remove after a maximum of 9 hours. Alternate between the right and left sides to avoid irritation. If the patch falls off, place a new patch on a different area of the same hip, but total

wear time should not exceed 9 hours from application of the first patch. The patch is applied for a total of 9 hours, but effects last at least 12 hours. Absorption increases with external heat (such as a heating pad, electric blanket, or heated water-bed) or when patch is applied to inflamed skin. The patches must not be cut as the effects on dose delivery have not been studied. Initiate treatment at the lowest patch dose, 10 mg, and titrate to effect on a weekly basis in both methylphenidate-naïve patients and when converting patients from oral methylphenidate agents regardless of previous dose. After removal, fold the patch so that no adhesive is exposed and flush or place in a lidded container.

Daytrana™ is supplied in 10mg, 15 mg, 20 mg, and 30 mg patches and comes in trays of 10 or 30. Store unused patches in their original pouch at room temperature. Use patches within 2 months of opening the tray.

Table 1. Cost Comparison of Once-Daily Methylphenidate Formulations

	Starting Dose	**AWP** 30-day supply	**WAC** 30-day supply
Daytrana™ (10 mg, 15 mg, 20 mg, 30 mg)	10 mg once daily	198.50	119.40
Concerta® (18 mg, 27 mg, 36 mg, 54 mg)	18 mg once daily	111.95	89.56
Ritalin LA® (10 mg, 20 mg, 30 mg, 40 mg)	20 mg once daily	92.69	74.15
Metadate CD™ (10 mg, 20 mg, 30 mg)	20 mg once daily	82.29	65.83

In summary, Daytrana™ is the first methylphenidate transdermal system to be approved for the treatment of ADHD in children ages 6-12 years old. Benefits of the patch include its once daily application, the ability to remove the patch early to minimize side effects, and its availability for children who are unwilling or unable to take oral medications. Due to risks of allergic contact dermatitis and methylphenidate sensitivity, it may not be first-line treatment, but will be available for patients unable or unwilling to take methylphenidate orally.

1. Daytrana™ (methylphenidate transdermal system) [package insert]. Wayne, PA: Shire Pharmaceuticals Ireland Limited, April 2006.
2. Anderson VR, Scott LJ. Methylphenidate transdermal system: in attention-deficit hyperactivity disorder in children. Drugs 2006;66(8):1117-26.
3. Fleming T. ed 2005 Redbook. Montvale, NJ: Thomson PDR; 2005.

EXHIBIT 4

Source: USPQ, 2d Series (1986 - Present) > U.S. Court of Appeals, Federal Circuit > Alza Corp. v. Mylan Laboratories Inc., 80 USPQ2d 1001 (Fed. Cir. 2006)

Alza Corp. v. Mylan Laboratories Inc., 80 USPQ2d 1001 (Fed. Cir. 2006)

80 USPQ2d 1001
Alza Corp. v. Mylan Laboratories Inc.
U.S. Court of Appeals
Federal Circuit
No. 06-1019
Decided September 6, 2006
464 F3d 1286

Headnotes

PATENTS

[1] Patentability/Validity — Obviousness — Person of ordinary skill in art (▶115.0902)

Patentability/Validity — Obviousness — Combining references (▶115.0905)

Under non-rigid "motivation-suggestion-teaching"test, suggestion to combine prior art references can be found in knowledge generally available to person of ordinary skill in art, as well as in references themselves, and expert testimony therefore is pertinent to evaluation of prima facie case of obviousness if such testimony is relevant to determining knowledge that person of ordinary skill in art would have possessed at given time; in present case, infringement defendants have established, by clear and convincing evidence, that invention of patent for extended-release oxybutynin formulation was rendered obvious by combination of prior art references, since record shows that teachings of references would have conveyed to person of ordinary skill, once motivated to use oxybutynin, reasonable expectation of success in manufacturing controlled release oxybutynin formulation, since testimony of defendant's expert supports finding that, based on oxybutynin's lipophilicity, person of skill in art would have had reasonable expectation that oxybutynin would be colonically absorbed and therefore would have been motivated to produce claimed extended release formulation, and since references cited by plaintiff are entirely consistent with that finding.

[2] Infringement — Literal infringement (▶120.05)

Plaintiff failed to establish that accused extended-release oxybutynin formulation infringed patent in suit, since patent specifically requires that time-course of in vivo oxybutynin release for claimed formulation fall within certain boundaries, since plaintiff presented evidence of blood plasma concentration-versus-time profiles for both accused formulation and embodiment of formulation claimed in patent, but, even if it is assumed that drug is rapidly taken up into bloodstream upon dissolution, there is no expert testimony or other evidence to show that plasma concentration-versus-time data establishes in vivo release rates for either accused formulation or patented embodiment, and since plaintiff's evidence of in vitro dissolution rates is irrelevant absent evidence demonstrating that in vitro system is good model of actual in vivo behavior; conclusion of noninfringement does not require specific finding that two bodies of evidence presented by plaintiff are inadequate when considered both individually and in

combination, since instant case does not present situation in which two pieces of otherwise severely inadequate evidence create great probative value synergistically.

Particular Patents

EXHIBIT 4

Particular patents — Chemical — Oxybutynin formulations

6,124,355, Guittard, Jao, Marks, Kidney, and Gumucio, oxybutynin therapy, judgment of invalidity and noninfringement affirmed.

Case History and Disposition

Appeal from the U.S. District Court for the Northern District of West Virginia, Keeley, C.J.

Action by Alza Corp. against Mylan Laboratories Inc. and Mylan Pharmaceuticals Inc. for patent infringement. Plaintiff appeals from judgment of invalidity and noninfringement following bench trial. Affirmed.

Attorneys

Gregory L. Diskant, Jeffrey I.D. Lewis, and Richard J. McCormick, of Patterson, Belknap, Webb & Tyler, New York, N.Y., for plaintiff-appellant.

John B. Wyss, James H. Wallace Jr., Kevin P. Anderson, and Robert J. Scheffel, of Wiley, Rein & Fielding, Washington, D.C., for defendants-appellees.

Judge

Before Gajarsa, circuit judge, Clevenger, senior circuit judge, and Prost, circuit judge.

Opinion Text

Opinion By:

Gajarsa, J.

Alza Corp. ("Alza") appeals from the district court's judgment, after a bench trial, of noninfringement and invalidity of claims 1-3, 11, 13 and 14 of U.S. Patent No. 6,124,355[1] ("the '355 patent") in favor of Mylan Laboratories, Inc. and Mylan Pharmaceuticals, Inc. (collectively, "Mylan"). *Alza Corp. v. Mylan Labs., Inc.*, 388 F.Supp.2d 717 (N.D.W. Va. 2005)("*Alza II*"). The infringement arose from Mylan's filing of two Abbreviated New Drug Applications ("ANDAs") for a generic version of the once-a-day extended release formulation of the anti-incontinence drug oxybutynin, *id.* at 720, which Alza has been marketing as Ditropan XL®. *Id.* at 738. This court has jurisdiction pursuant to 28 U.S.C. § 1295(a)(1). For the reasons stated below, we affirm the district court's judgment of noninfringement and invalidity.

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[1] The '355 patent issued to Guittard et al. and was assigned to Alza.

I. BACKGROUND

This litigation arose from Mylan's and Impax's filings of ANDAs for once-daily, controlled-release oxybutynin formulations. Oxybutynin is a drug used to treat urinary incontinence. Once-a-day dosing provides the usual benefits of convenience, steady-dosing, and in addition, possibly reduced absorption of a metabolite that leads to side-effects. Claim 2 of the '355 patent is representative.

2. A sustained-release oxybutynin formulation for oral administration to a patient in need of treatment for urge incontinence comprising a therapeutic dose of an oxybutynin selected from the group consisting of oxybutynin and its pharmaceutically acceptable salt that *delivers* from 0 to 1 mg in 0 to 4 hours, from 1 mg to 2.5 mg in 0 to 8 hours, from 2.75 to 4.25 mg in 0 to 14 hours, and 3.75 mg to 5 mg in 0 to 24 hours for treating urge incontinence in the patient.

col. 17, ll. 31-38 (emphasis added).

The district court construed the '355 patent claims in its *Markman* Order, reported at *Alza Corp. v. Mylan Labs., Inc.*, 349 F.Supp.2d 1002 (N.D.W. Va. 2004) ("*Alza I*"). The court construed the word "deliver" to refer to the rate of *in vivo* release in the gastrointestinal ("GI") tract. *See id.* at 1019.

Alza did not present direct evidence that Mylan's ANDA formulation released drug in the GI tract at the rates claimed by the '355 patent. However, it did offer two other types of evidence: 1) the rate at which the generic product released oxybutynin in an *in vitro* dissolution apparatus, and 2) the rate at which the ANDA product resulted in the accumulation of oxybutynin in the bloodstream.

The district court found that Alza had failed to meet its burden of proof on infringement. The district court also found the asserted claims of the '355 patent to be invalid as both anticipated and obvious in light of the prior art. For the reasons stated below, we affirm the invalidity holding on obviousness grounds, and consequently, we do not need to reach Alza's arguments regarding anticipation. We also affirm the holding of noninfringement.

II. DISCUSSION

A. Standard of review

Infringement is a question of fact that, after a bench trial, we review for clear error. *See,*

e.g., *Ferguson Beauregard/Logic Controls, Div. of Dover Res., Inc. v. Mega Sys., LLC*, 350 F.3d 1327, 1338 [69 USPQ2d 1001] (Fed. Cir. 2003). Under the clear error standard, a reversal is permitted only when this court is left with a definite and firm conviction that the district court was in error. *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1164 [77 USPQ2d 1865] (Fed. Cir. 2006).

As for obviousness, a claimed invention is unpatentable if the differences between it and the prior art are "such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103(a) (2000); *In re Kahn*, 441 F.3d 977, 985 [78 USPQ2d 1329] (Fed. Cir. 2006) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 13-14, [148 USPQ 459] (1966)). Obviousness is a question of law, reviewed *de novo*, based upon underlying factual questions which are reviewed for clear error following a bench trial. *Ruiz v. A.B. Chance Co.*, 357 F.3d 1270, 1275 [69 USPQ2d 1686] (Fed. Cir. 2004). These "underlying factual inquiries includ[e]: (1) the scope and content of the prior art; (2)the level of ordinary skill in the prior art; (3) the differences between the claimed invention and the prior art; and (4) objective evidence of nonobviousness." *In re Dembiczak*, 175 F.3d 994, 998 [50 USPQ2d 1614] (Fed. Cir. 1999). Similarly, "[t]he presence or absence of a motivation to combine references in an obviousness determination is a pure question of fact," *In re Gartside*, 203 F.3d 1305, 1316 [53 USPQ2d 1679] (Fed. Cir. 2000); *accord Winner Int'l Royalty Corp. v. Wang*, 202 F.3d 1340, 1348 [53 USPQ2d 1580] (Fed. Cir. 2000), as is the presence or absence of a "reasonable expectation of success" from making such a combination, *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1165 [77 USPQ2d 1865] (Fed. Cir. 2006). Because "a patent retains its statutory presumption of validity, *see* 35 U.S.C. § 282, ... the movant retains the burden to show the invalidity of the claims by clear and convincing evidence as to underlying facts." *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1349 [60 USPQ2d 1001] Fed. Cir. 2001)(internal quotations omitted).

In *Graham*, the Court held that that the obviousness analysis begins with several basic factual inquiries:

"[(1)] the scope and content of the prior art are to be determined; [(2)] differences between the prior art and the claims at issue are to be ascertained; and [(3)] the level of ordinary skill in the pertinent art resolved." 383 U.S. at 17. After ascertaining these facts, the Court held that the obviousness *vel non* of the invention is then determined "against th[e] *background*'of the *Graham* factors. *Id.* at 17-18 (emphasis added). Clearly, the Court recognized the importance of guarding against hindsight, as is evident in its discussion of the role of secondary considerations as "serv[ing]to guard against slipping into use of hindsight and to resist the temptation to read into the prior art the teachings of the invention in issue." *Id.* at 36.

The Court of Appeals for the Federal Circuit's and its predecessor's "motivation to combine" requirement likewise prevents statutorily proscribed hindsight reasoning when determining the obviousness of an invention. *Kahn*, 441 F.3d at 986 ("[T]he 'motivation-suggesting-teaching'requirement protects against the entry of hindsight into the obviousness analysis."); *In re Fridolph*, 30 CCPA 939, 942 (1943) ("[I]n considering more than one reference, the question always is: does such art suggest doing the thing the [inventor] did."). According to the "motivation-suggesting-teaching"test, a court must ask "whether a person of ordinary skill in the art, possessed with the understandings and knowledge reflected in the prior art, and motivated by the general problem facing the inventor, would have been led to make the combination recited in the claims." *Kahn*, 441 F.3d at 988 (citing *Cross Med. Prods., Inc., v. Medtronic Sofamor Danek, Inc.*, 424 F.3d 1293, 1321-24 [76 USPQ2d 1662] (Fed. Cir. 2005)).

This requirement has been developed consistent with the Supreme Court's obviousness jurisprudence as expressed in *Graham* and the text of the obviousness statute that directs us to conduct the obviousness inquiry "at the time the invention was made" 35 U.S.C. § 103. As we explained in *Kahn*,

> The motivation-suggestion-teaching test picks up where the analogous art test leaves off and informs the *Graham* analysis. To reach a non-hindsight driven conclusion as to whether a person having ordinary skill in the art at the time of the invention would have viewed the subject matter as a whole to have been obvious in view of multiple references, the Board must provide some rationale, articulation, or reasoned basis to

explain why the conclusion of obviousness is correct. The requirement of such an explanation is consistent with governing obviousness law ... .

441 F.3d at 987. We further explained that the "motivation to combine" requirement "[e]ntails consideration of both the 'scope and content of the prior art'and 'level of ordinary skill in the pertinent art' aspects of the *Graham* test." *Id.* at 986.

At its core, our anti-hindsight jurisprudence is a test that rests on the unremarkable premise that legal determinations of obviousness, as with such determinations generally, should be based on evidence rather than on mere speculation or conjecture. Our court's analysis in *Kahn* bears repeating:

> A suggestion, teaching, or motivation to combine the relevant prior art teachings *does not have to be found explicitly in the prior art*, as "the teaching, motivation, or suggestion may be implicit from the prior art as a whole, rather than expressly stated in the references... . The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art." However, rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be *some* articulated reasoning with *some* rational underpinning to support the legal conclusion of obviousness. This requirement is as much rooted in the Administrative Procedure Act [for our review of Board determinations], which ensures due process and non-arbitrary decisionmaking, as it is in § 103.

441 F.3d at 987-88 (quoting *In re Kotzab*, 217 F.3d 1365, 1370 [55 USPQ2d 1313] (Fed. Cir. 2000)) (citations omitted) (emphases added)). There is flexibility in our obviousness jurisprudence because a motivation may be found *implicitly* in the prior art. We do not have a rigid test that requires an actual

teaching to combine before concluding that one of ordinary skill in the art would know to combine references. This approach, moreover, does not exist merely in theory but in practice, as well. Our recent decisions in *Kahn* and in *Cross Medical Products* amply illustrate the current state of this court's views. *See Kahn*, 441 F.3d at 988 (affirming the PTO's obviousness finding, explaining that a motivation to combine may be found in implicit factors, such as the "knowledge of one of ordinary skill in the art, and [what] the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art"); *Cross Med. Prods.*, 424 F.3d at 1322 (reversing a district court ruling of nonobviousness and explaining that "the motivation to combine need not be found in prior art references, but equally can be found in the knowledge generally available to one of ordinary skill in the art"such as knowledge of a problem to be solved).

In conclusion, our approach has permitted us to continue to address an issue of law not readily amenable to bright-line rules, as we recall and are guided by the wisdom of the Supreme Court in striving for a "practical test of patentability." *Graham*, 383 U.S. at 17.

B. Description of the technology

The patent at issue is directed generally to an extended release form of oxybutynin. Because the subject matter of the patent falls roughly under the rubric of pharmacology, we give a brief orientation to the field, based upon the record. In general, when a drug is swallowed, it is (1) dissolved in the gastrointestinal("GI") tract; (2) absorbed from the GI tract into the bloodstream;(3) distributed from the blood into body tissues; and (4) metabolized and eliminated from the bloodstream. The GI tract includes the stomach, small intestine and the colon, and orally administered drugs pass through these portions of the GI tract in turn. Drugs may be administered in different dosage forms,[2] which may include not only the drug itself but also ingredients intended to modulate the rate of release of the drug from the dosage form.

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[2] Here we are discussing oral dosage forms, specifically.

Dosage forms may be described as immediate-release, e.g., such as where the drug is quickly released in the stomach, or as sustained/extended-release, where the drug is slowly released as the formulation traverses the GI tract. The rate of absorption of a drug from the GI tract into the bloodstream may change as it passes through the GI tract. The rate of absorption for a dissolved drug in a given portion of the GI tract also varies from drug to drug.

After roughly 8-12 hours a typical dosage form will reach the colon. If, hypothetically, a

particular drug is simply not absorbed from the colon into the bloodstream, then it may make little sense to develop an extended-release dosage form that is capable of "withholding"the release of some fraction of that drug until it reaches the colon. In other words, under these hypothetical conditions, there may be little motivation to design an oral dosage form capable of releasing drug more *slowly* than over an approximately 8-12 hour time course, because such drug would be released in the colon, where it is (hypothetically) not absorbed.

The '355 patent claims an extended release oxybutynin formulation. Alza argues that one of ordinary skill in the art would not have believed that oxybutynin could be absorbed in the colon. Absent such absorption, Alza contends that one of ordinary skill in the art lacked the motivation to make the claimed extended release formulation, and that the district court therefore erred in holding that the asserted claims are invalid as obvious over the prior art. For the reasons set forth below, Alza's arguments fail.

C. Invalidity

EXHIBIT 4

The district court based its invalidity holding both on anticipation and obviousness grounds. Because we affirm its holding based on obviousness, we do not need to address the parties' anticipation arguments.

In finding the asserted claims of the '355 patent to be obvious, the district court considered, *inter alia*, the following prior art: U.S Patent Nos. 5,399,359 ("the Baichwal patent"); 5,082,688 ("the Wong patent"); and 5,330,766 ("the Morella patent").

The Morella patent discloses a "sustained-release pharmaceutical composition including an active ingredient of high solubility in water ... ."According to the specification, highly soluble drugs had posed special challenges for the development of sustained release forms, which the inventors had set out to solve. "Sustained-release" is defined as release of the active ingredient at a rate that maintains therapeutic, nontoxic blood levels "over an extended period of time e.g. 10 to 24 hours or greater." Highly water soluble drugs were considered to be those having an aqueous solubility of at least roughly 1 part in 30. The commercially available hydrochloride salt of oxybutynin is freely soluble at neutral pH. The patent uses morphine as an example of an active ingredient that can be used in its compositions. Figure 5 demonstrates that one such composition is capable of dispensing morphine at what appears to be an approximately steady rate over the course of 24 hours. Claim 2 of the patent claims "genitourinary smooth muscle relaxants"as one of several types of active ingredients to use in the dosage form identified in claim 1. The specification also identifies oxybutynin as a highly water soluble genitourinary smooth muscle relaxant. Morella also teaches that "the dissolution rate of the soluble drug at various pH's can be modified at will."

The Baichwal patent teaches a 24 hour extended release oxybutynin formulation. These formulations use an enteric-coated polymer matrix similar to Mylan's accused product. It also teaches methods of modifying the dosage forms to slow the release rates. During prosecution of the '355 patent, the inventor overcame an anticipation rejection by arguing that his invention had a release rate slower than those of the dissolution data presented in Baichwal.[3] The examiner agreed and withdrew his rejection.

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[3] Tables 15 and 18 of Baichwal, for example, disclose *in vitro* dissolution rates in which roughly half of the drug is dissolved by four hours.

The Wong patent teaches a bilayer osmotic pump dosage form ("the OROS system")used in the preferred embodiment of the '355 patent. Wong teaches that this system can be used to deliver any drug over a 24 hour period, and Figure 11 of the patent discloses release rates falling within the claimed release rates of the '355 patent. The Wong patent does not specifically teach using oxybutynin with the claimed release technology, but it does teach using several categories of drugs of which oxybutynin is a member, such as anti-cholinergics, analgesics, muscle relaxants and urinary tract drugs.

In analyzing the obviousness issue, the district court first identified the level of ordinary skill in the art, finding the person of ordinary skill to have either an advanced degree in pharmacy, biology, chemistry or chemical engineering and at least two years of experience with controlled-release technology; or a bachelor's degree in one (or more) of those fields plus five years of experience with such technology. Second, the court examined whether there was a motivation "in the prior art or elsewhere that would have led one of the ordinary

skill in the art to combine references," *Alza II*, 388 F.Supp.2d at 737 (citing *Ruiz*, 234 F.3d at 664 (internal quotations omitted)), and with a "reasonable expectation of success," *id.* (citing *In re O'Farrell*, 853 F.2d 894, 904 [7 USPQ2d 1673] (Fed. Cir. 1988)). Third, the district court examined secondary considerations of nonobviousness. After making these factual determinations, it concluded that Mylan had established a

strong prima facie case of obviousness, which Alza had failed to rebut through secondary considerations. The court concluded that Mylan had demonstrated Alza's patent to be invalid for obviousness by clear and convincing evidence.[4] We agree.

[4] Having reviewed Alza's sundry contentions that the district court made findings inconsistent with the appropriate burdens of proof for infringement and invalidity, we find them to be without merit.

[1] While we have carefully considered all of the parties'arguments, we discuss principally the dispute over satisfaction of one predicate to a finding of obviousness: that a person of ordinary skill in the art would have had a "motivation to combine" the prior art to achieve the claimed invention and that she would have had a "reasonable expectation of success" in doing so. As an initial matter, we agree with the district court that "on a purely mechanical level, a person of ordinary skill in the art would have a reasonable expectation of success of manufacturing a 24 hour controlled-release oxybutynin formulation ... . *once motivated to use oxybutynin.*" *Id.* at 739. For example, Wong teaches a rate adjustable extended release dosing technology and release rates falling within the claimed parameters. Baichwal and Wong likewise teach ways of achieving slow rates of release, with Baichwal actually teaching extended-release oxybutynin, although arguably not as slowly as is claimed in the '355 patent.[5]

[5] The patent examiner initially rejected the '355 patent as anticipated by Baichwal, but subsequently allowed its issuance.

Indeed, Alza's principal argument is that no one of ordinary skill in the art would have been motivated to adapt the Morella, Baichwal and Wong technology to oxybutynin *in the first place*, because a person of ordinary skill in the art would have had no reason to expect that such an extended release oxybutynin formulation would have therapeutic value. The issues, as explained above, reduce essentially to whether one of ordinary skill in the art in 1995 would have had a reasonable expectation that oxybutynin would be colonically absorbed and therefore would have been motivated to produce the claimed extended release formulation.

The district court concluded that "the weight of the evidence clearly and convincingly establishes that a person of ordinary skill in the art in 1995 would reasonably expect oxybutynin to absorb in the colon ... [and] have a reasonable expectation of success of producing a 24 hour oxybuytnin formulation meeting the claims of the '355 patent."[6] *Alza II*, 388 F.Supp.2d at 740. Alza argues, however, that the district court erred because " *[t]here was no prior art evidence supporting this finding.*" According to Alza, "[t]here was no contemporaneous documentation supporting the view that any one factor—lipophilicity or anything else—existed to identify successful candidates for once-a-day delivery." It also argues that two prior art references "decisively undercut" the opinion of Mylan's expert, Dr. Amidon, which the district court cited in support of its conclusion. *See Alza II*, 388 F.Supp.2d at 739-740.

[6] The '355 patent issued on September 26, 2000 and claimed priority as far back as 1995. *See* '355 patent, col. 1, ll. 5-12. The district court treated 1995 as the relevant date for the obviousness inquiry, *see* Alza II, 388 F.Supp.2d at 740, as do both parties in their obviousness arguments before this court. *See, e.g.*, Alza Reply Br. at 13 (stating that "[t]he dispositive obviousness issue was whether colonic absorption of oxybutynin was reasonably expected in *1995*") (emphasis added); Mylan Br. at 6 & n.2 (referring to evidence establishing "the clear expectation of one skilled in the art in *1995*" and noting in a footnote that 1995 is "[t]he earliest possible date to which Alza asserts priority.")(emphasis added).

EXHIBIT 4

As an initial matter, it is essential to recognize that, as we have explained above, under our non-rigid "motivation-suggesting-teaching" test, a suggestion to combine need not be found in the prior art. *See Cross Med. Prods.*, 424 F.3d at 1322 ("[T]he motivation to combine need not be found in prior art references, but equally can be found in the knowledge generally available to one of ordinary skill in the art ... ."). Accordingly, where the testimony of an expert witness is relevant to determining the knowledge that a person of ordinary skill in the art would have possessed at a given time, this is one kind of evidence that is pertinent to our evaluation of a *prima facie* case of obviousness. We now

turn to consider whether the relevant evidence, including the expert testimony and the prior art, when viewed as a whole supports the findings of the district court. We conclude that the findings of the district court were not clearly erroneous.

Mylan's expert, Dr. Amidon, testified that based on its lipophilicity, he would "expect oxybutynin to be a highly permeable" compound that is "rapidly absorbed" along the length of the GI tract, including the colon. Later, when challenged about the predictive value of lipophilicity, Dr. Amidon explained, "I would say there were some unknowns, but again lipophilic drugs would be well absorbed. That would be—that was the general understanding at the time."

Although Alza argues that two prior art references "decisively undercut Dr. Amidon's hindsight opinion," these references are in fact not inconsistent with the general principle that the extent of a drug's colonic absorption correlates with its lipophilicity. Indeed, the first reference, a 1990 publication in the Journal of Pharmaceutical Sciences, states that "[i]n general, the more lipophilic drugs were transported rapidly." P. Artursson, *Epithelial Transport of Drugs in Cell Culture. I: A Model for Studying the Passive Diffusion of Drugs over Intestinal Absorptive (Caco-2) Cells*. 79 J. Pharm. Sci. 476, 481 (1990). Alza, however, cites this reference narrowly for its observation that a highly lipophilic analog of a particular drug did not follow the general rule that lipophilic drugs were transported more quickly. *Id.* Granted, the authors admit that "[t]he reason for this [deviation]is currently unknown," and they postulate that it may be related to a physicochemical factor other than lipophilicity, namely steric hindrance.[7] *Id.* But the mere fact that the colonic absorption rate of a drug may be predicted most precisely by using "many factors,"rather than "lipophilicity" alone, does not negate the general predictive utility of lipophilicity in estimating the extent of colonic absorption.

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[7] Dr. Chancellor, Alza's expert, likewise characterized colonic absorption as having been understood as being dependent on several physicochemical and physiological variables, of which lipophilicity was one.

The second prior art reference cited by Alza, *Absorption of Polar Drugs Following Caecal Instillation in Healthy Volunteers*, is similarly unavailing to it. Riley, et al., 6 Aliment. Pharmacol. Ther. 701, 705 (1992). Again, this reference teaches that while the correlation is not perfect, lipophilicity tended to suggest colonic absorption, stating that "[t]he relationship between the physical characteristics of a drug and its colonic absorption is not yet clear but studies in the rat suggest that *lipophilic drugs are well absorbed along the length of the gastrointestinal tract*, whereas hydrophobic polar drugs are absorbed much less from the colon than from the small intestine." *Id.* (emphasis added).

Far from teaching away or detracting from the weight of Dr. Amidon's testimony, these prior art references, taken as a whole, are entirely consistent with the finding that in 1995 a person of ordinary skill in the art would have expected a general, albeit imperfect, correlation between a drug's lipophilicity and its colonic absorptivity. Accordingly, we cannot perceive clear error in the district court's factual findings that

while colonic absorption was not *guaranteed*, the evidence, viewed as a whole, is clear and convincing that a person of ordinary skill in the art would nonetheless have perceived a reasonable likelihood of success and that she would have been motivated to combine prior art references to make the claimed invention.

Likewise, we find no error in the district court's consideration of secondary indicia of obviousness. We therefore affirm its legal conclusion of obviousness, finding the district court to have correctly held that Mylan met its burden of overcoming the presumption of validity that attaches to an issued patent.

D. Infringement

The '355 patent specifically describes the rate of oxybutynin release from its "extended release" formulations, requiring that the time-course of *in vivo* oxybutynin release falls within certain boundaries. That is, at certain times, the cumulative amount of dissolved (released) drug must fall within certain ranges. To prove infringement, Alza bore the burden of proving, *inter alia*, that Mylan's accused generic formulation exhibited an *in vivo* release profile falling within the claimed ranges at the relevant times.

At trial, Alza presented no direct evidence of how quickly the accused product dissolved *in vivo*. *Alza II*, at 722. However, it did offer two kinds of indirect evidence as measures of the rate of *in vivo* release. *Id.* First, it presented

evidence of the blood plasma concentration versus time profiles for both the accused ANDA formulation and Ditropan, an embodiment of the '355 patent. Second, it presented evidence of the rate of release not in the GI tract but in pieces of laboratory apparatus under certain experimental conditions, so-called *in vitro dissolution*. The critical deficiency in the evidence presented by Alza was not that it was "indirect" rather than "direct,"but rather that it failed to credibly link these pieces of evidence with the relevant pharmacokinetic parameter—the rate of *in vivo* dissolution in the GI tract.

Thus, the district court explained that Alza had failed to demonstrate how evidence of the rate of dissolution of drug in the GI tract could be extracted from plots of plasma concentration versus time. The district court accepted Alza's simplifying assumption about oxybutynin rapidly being absorbed following dissolution such that the rates of *in vivo* dissolution parallel the rate of drug uptake into the blood. However, it found that only one expert, Dr. Amidon, had "endorsed Alza's subjective comparison of blood plasma levels with *in vivo* release rates." As for that one expert, moreover, he "rejected the very conclusion that Alza attributed to him."

[2] Alza criticizes the district court for "fail[ing]to come to grips with the significance of the testimony" that Dr. Amidon "recanted ...immediately after he made it." Specifically, Alza urges that notwithstanding the expert's recantation, we should nonetheless draw our independent conclusions from the "point of his testimony" that release rates in blood and the appearance in the GI tract are essentially the same. We have considered Alza's arguments and find them to lack legal and factual coherency. Even if we were to presume to be experts and to apply the simplifying assumption that the drug is rapidly taken up into the bloodstream upon dissolution, it is not clear to us how to abstract from each plasma concentration versus time curve the rate of uptake into the bloodstream. This would require factoring out of the curve the effects, *inter alia*, of the elimination of drug from the bloodstream over the relevant 24 hour period. But this is not our province. Such evidence, if it exists, must have been presented at trial, or in its stead other evidence sufficient to persuade the trial court.

From what can be discerned, Dr. Amidon's immediately recanted statement appears to have been based on his comparison of the relative areas under the curves of plasma concentration versus time plots of both the accused ANDA formulation and Ditropan XL. Indeed, Alza reproduces in its appellate brief Dr. Amidon's testimony that the accused product has only 92 to 93 percent of the area under the curve of Ditropan XL. This appears to have resulted in the drawing of a line (referred to by the parties as "line A") on a plot of *in vitro* dissolution of both Ditropan XL and the accused ANDA formulation, wherein the rate

of *in vitro* dissolution of Mylan's ANDA formulation has been adjusted according to that percentage. The basis for, and significance of, line A is simply not apparent from the record, and Alza fails to provide us with any persuasive line of argument as to how we should imbue line A with any relevant meaning. In short, we agree with Mylan that the plasma concentration versus time data fail to establish *in vivo* release rates for either Ditropan XL or the accused ANDA product.

The district court similarly found unpersuasive Alza's evidence that Ditropan XL and the accused ANDA product sometimes exhibited *in vitro* dissolution rates falling within the claims. The court cited testimony by Dr. Amidon explaining that these *in vitro* procedures are "not designed to reflect the *in vivo* dissolution process." This accords with the common sense notion that one cannot simply proclaim without proof that he has constructed an apparatus capable of mimicking pertinent environmental variables of the GI tract (along the length of the tract, nonetheless). Indeed, the obtained *in vitro* dissolution rates vary widely with the choice of experimental parameters. We agree with the district court that Alza's evidence of *in vitro* dissolution rates is irrelevant absent evidence demonstrating that the *in vitro* system is a good model of actual *in vivo* behavior. On that point, Alza's evidence is severely lacking.

We therefore affirm the district court's finding of noninfringement. In so doing we explicitly reject Alza's suggestion that the district court erred in failing to specifically state that not only did it find Alza's plasma concentration data and its "*in vitro*" data to be inadequate in isolation, but that it had also found the data to be inadequate in combination. Even if we were to entertain the suggestion

that the district court was in fact unfamiliar with the basic precept that it is the totality of the evidence that it must consider in making factual determinations, we would merely conclude that where as here, if each of two pieces of evidence, assessed separately, is severely inadequate to support a proposition, when their probative values are tallied, they still fall short. While it is possible to envision cases in which two pieces of evidence may create great probative value synergistically, this is not one of those cases.

* * *

In conclusion, we affirm the judgment of the district court that the asserted claims of the '355 patent were invalid, and that notwithstanding, the patent was not infringed.

AFFIRMED.

Costs to Mylan.

- End of Case -

EXHIBIT 5

Pergamon

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Lack of Effect of Repeated Administration of Milnacipran, a Double Noradrenaline and Serotonin Reuptake Inhibitor, on the β-adrenoceptor-linked Adenylate Cyclase System in the Rat Cerebral Cortex

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Summary—The effects of subacute administration of the double noradrenaline and serotonin uptake inhibitor antidepressant, milnacipran, and the tricyclic antidepressant, imipramine, on radioligand binding to β-adrenergic receptors and on β-adrenergic agonist-stimulated adenylate cyclase activity, in the rat cerebral cortex, have been determined. Rats were injected intraperitoneally for 21 days with milnacipran (3, 10 or 30 mg/kg/day) or imipramine (10 mg/kg/day). The treatment with milnacipran up to 30 mg/kg/day did not modify either the maximum number of [^{3}H]CGP-12177 binding sites (B_{max}) or the equilibrium dissociation constant (K_d). On the other hand, treatment of the rats with 10 mg/kg/day imipramine induced a decrease (27%) in B_{max} [^{3}H]CGP-12177 binding sites without affecting the K_d value. Furthermore, milnacipran did not affect the stimulation of cAMP production induced by either 30 μM isoprenaline, 10 μM GTPγS or 10 μM forskolin. Under similar conditions, treatment with imipramine reduced by 70% the isoprenaline-induced stimulation of cAMP production without affecting that induced by either GTPγS or forskolin. These results demonstrate that, unlike imipramine, subacute administration of milnacipran does not produce any change in β-adrenoceptor sensitivity in the rat brain cortex. Copyright © 1996 Elsevier Science Ltd.

Keywords—Milnacipran, imipramine, β-adrenoceptor binding, β-adrenoceptor-linked adenylate cyclase, antidepressants, adenylate cyclase.

All known antidepressants take 2–3 weeks to manifest their antidepressant activity in man although their biochemical effects, such as the inhibition of monoamine uptake, are apparent within hours. The fact that most clinically effective antidepressants decrease the density of β-adrenoceptors and the response of adenylate cyclase to β-adrenoceptor agonists after repeated administration for 2–3 weeks has led to the idea that down-regulation of β-adrenoceptors in the brain may be involved in the mechanism of action of antidepressant drugs (Sulser *et al.*, 1983).

While certain selective serotonin reuptake inhibitors (SSRIs), such as paroxetine (Nelson *et al.*, 1989), fluvoxamine (Benfield and Ward, 1986) and citalopram (Hyttel *et al.*, 1984), appear clearly not to down-regulate β-adrenoceptors, all noradrenaline uptake inhibiting antidepressants so far studied down-regulate β-adrenoceptors. Although Barbaccia *et al.* (1986) failed to observe any down-regulation with the selective noradrenaline uptake inhibitor, maprotiline, others (Olpe and Schellenberg, 1980; Schoffelmeer *et al.*, 1984) have reported down-regulation. Similarly, the findings with the noradrenaline and dopamine uptake inhibitor, buproprion, are controversial with some groups not finding down-regulation (Ferris and Beaman, 1983) while others did (Gandolfi *et al.*, 1983).

The only noradrenaline uptake inhibiting antidepressant that has been unequivocally shown not to down-regulate β-adrenoceptors is milnacipran (midalcipran; F 2207). This double noradrenaline and serotonin uptake inhibitor (Moret *et al.*, 1985; Stenger *et al.*, 1987) has been shown to be an effective antidepressant with equivalent efficacy to amitriptyline (Ansseau *et al.*, 1989). We (Moret *et al.*, 1985; Assié *et al.*, 1992) and

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others (W. Buckett, personal communication; Carré *et al.*, 1988; Matsubara *et al.*, 1988, 1990) have been unable to detect any desensitisation of the β-adrenoceptors even though a wide range of doses and administration schedules have been tested. To date, however, the lowest dose studied has been 15 mg/kg/day which is relatively high compared to doses required for activity in tests predictive of antidepressant activity (3–10 mg/kg po) such as the forced swimming paradigm in mice, the learned helplessness test in rats (unpublished data) and the bulbectomised rat (Redmond *et al.*, 1995). In addition, relatively little attention has been paid to the desensitisation of adenylate cyclase although the lack of down-regulation of adenylate cyclase was reported in an abstract (Matsubara *et al.*, 1990).

We report here a study of the effect of repeated administration of milnacipran for 3 weeks at three doses, covering the active range of milnacipran in tests of antidepressant activity (3–30 mg/kg/day) on β-adrenoceptor binding and β-adrenoceptor-linked adenylate cyclase activity.

METHODS

Animals

Adult male Wistar rats (CERJ, Saint Berthevin, France), weighing 160–190 g, were used for this study. They were housed in group cages (6 animals receiving the same treatment in the same cage) under standard conditions (21–23°C, 12-hr light–dark cycle) with free access to food and water. Animals were weighed before treatment and on days 3, 7, 10, 14, 17 and 21 of treatment.

Drug treatments

Thirty rats were housed for 5 days before their utilisation. They were then separated into five groups of 6 animals as follows:

Group:	Treatment:
1	saline solution (0.9% NaCl)
2	milnacipran (3 mg/kg/day)
3	milnacipran (10 mg/kg/day)
4	milnacipran (30 mg/kg/day)
5	imipramine (10 mg/kg/day).

Compounds were administered intraperitoneally in a volume of 0.5 ml/200 g in 0.9% NaCl, twice daily (08:00 and 18:00) for 21 days. All animals were killed by decapitation 24 hr after the last administration.

Membrane preparation

Membranes were prepared as described by Mork and Geisler (1989). Immediately after sacrifice, brains were removed into ice-cold saline (0.9% NaCl) and the cortex were rapidly dissected and weighed. Cortex were then homogenized using a Teflon-glass homogenizer (8 strokes, speed 10) in 10 volumes of cold buffer containing 320 mM sucrose, 2 mM Tris–maleate and 2 mM EGTA–Tris, pH 7.4. The homogenates were diluted in 2 mM Tris–maleate and 2 mM EGTA–Tris, pH 7.4, to give a final protein concentration of 2.4 mg/ml. These preparations were used immediately for adenylate cyclase assays or stored at −70°C until the day of experiment for binding assays.

Binding assays

Binding assays were carried out as described by Riva and Creese (1989) using the selective β-adrenoceptor radioligand [^{3}H](−)CGP-12177. After thawing, 1 ml of diluted membrane suspension containing 500 μg protein was added to 800 μl of incubation buffer (50 mM Tris–HCl, pH 7.4), 100 μl [^{3}H](−)CGP-12177, and 100 μl incubation buffer or alprenolol (final concentration, 10 μM) to measure the non-specific binding, in a total assay volume of 2 ml. After 30 min incubation at 37°C, the content of each tube was rapidly filtered under vacuum through Whatman GF/C filters, and then washed three times with 5 ml ice-cold Tris–HCl buffer using a Brandel cell harvester. Filters were placed in scintillation vials and 4 ml of scintillation liquid (Formula 989, DuPont NEN) were added. Bound radioactivity was determined using a liquid scintillation counter (LS 6000, Beckman).

Adenylate cyclase assays

Adenylate cyclase activity was measured by determination of [^{32}P]cAMP converted from [α-^{32}P]ATP as described by Salomon *et al.* (1974). Fresh preparations (120 μg protein/tube) were incubated in a buffer containing 0.2 mM ATP, 3 mM $MgCl_2$, 1 mM EGTA–Tris, 80 mM Hepes–Tris, pH 7.4, 0.5 mM IBMX, 5 mM phosphoenol pyruvate, 0.5 mg/ml pyruvate kinase, 10 μM GTP and 2 μCi [α-^{32}P]ATP. After a 10-min incubation at 30°C, the reaction was stopped by addition of 2% SDS, 40 mM ATP and 1.4 mM cAMP. [^{3}H]cAMP (20,000 dpm) was added to each tube as an internal control to calculate the recovery of cAMP. The samples were applied to 1 ml Dowex AG 50WX8 columns which were then washed with 2 × 1 ml H_2O and eluted with 3 ml H_2O. The eluate was supplemented with 0.2 ml imidazole (1.5 M) and applied to a neutral alumine column pre-equilibrated with imidazole. This eluate was collected into scintillation vials and 14 ml of scintillation liquid (Flow scint III, Packard) were added. Radioactivity was determined in a double-labelling (^{3}H and ^{32}P) mode using a liquid scintillation counter (LS 1701, Beckman).

Drugs used

[^{3}H](−)CGP-12177 and [^{3}H]cAMP were from DuPont NEN France, Les Ulis, France; [α-^{32}P]ATP from Amersham France, Les Ulis, France. Milnacipran hydrochloride was synthesized by Pierre Fabre Médicament. All other compounds were obtained from Sigma.

Data analysis

Saturation binding curves were obtained by incubating

[^{3}H](−)CGP-12177 over a concentration range of 0.01–2.5 nM with membrane protein in the absence (total binding) or presence of 10 μM alprenolol (non-specific binding). The specific binding was defined as the difference between total binding and non-specific binding. Determinations were made in duplicate. The dissociation constant (K_d) of [^{3}H](−)CGP-12177 and the maximum number of binding sites (B_{max}) were calculated by saturation curve fitting.

Preparations from each animal were stimulated or not by either 30 μm (−)isoprenaline, 10 μM GTPγS or 10 μM forskolin and cAMP production was measured. All determinations were made in triplicate or in quadruplicate. The results were expressed as a percentage of the basal level (in the absence of stimulation) after subtracting the background. For both assays, values presented are means ± SEM of six independent experiments. Statistical significance of differences was determined using unpaired Student's t-test.

RESULTS

Animal body weight variations

Neither treatments by milnacipran nor imipramine altered the body weight gain measured during the 21 day treatment.

Binding assays

In all cases Scatchard plots were linear indicating a single population of binding sites. In preparations from animals treated with milnacipran at 3, 10 or 30 mg/kg/day, neither the K_d for [^{3}H](-)CGP-12177 nor B_{max} values were significantly different from controls (Table 1). In preparations from animals treated with imipramine at 10 mg/kg/day, B_{max} was decreased by about 27% ($p < 0.01$) whereas the K_d value was not significantly modified (Table 1).

Adenylate cyclase assays

In preparations from saline-treated animals, isoprenaline (30 μM) significantly stimulated adenylate cyclase activity by a factor of 1.2. GTPγS and forskolin (10 μM each) stimulated the activity by factors of 3.7 and 15.7, respectively. In preparations from animals treated with milnacipran at 3, 10 or 30 mg/kg/day, the increase in adenylate cyclase activity induced by isoprenaline, GTPγS or forskolin was not significantly modified compared to respective control values (Table 2). In preparations from animals treated with imipramine at 10 mg/kg/day, the isoprenaline-induced increase in adenylate cyclase activity was significantly reduced by 70% ($p < 0.02$) whereas that induced by GTPγS or forskolin was not affected (Table 2).

Table 1. Effects of repeated administration of milnacipran and imipramine on β-adrenoceptor binding in the rat cortex

		B_{max} (fmol/mg protein)	K_d (nM)
Control		30.1 ± 0.7	0.121 ± 0.004
Milnacipran	(3 mg/kg/day)	31.7 ± 2.1	0.113 ± 0.003
	(10 mg/kg/day)	31.8 ± 1.5	0.117 ± 0.011
	(30 mg/kg/day)	30.4 ± 1.6	0.121 ± 0.006
Imipramine	(10 mg/kg/day)	22.1 ± 1.6*	0.122 ± 0.005

Values are mean ± SEM of six animals/group. * $p < 0.01$ compared to corresponding control values using the unpaired Student's t-test.

DISCUSSION

Milnacipran inhibits the reuptake of noradrenaline and serotonin while presenting no affinity (>10^{-5} M) for any neurotransmitter receptor studied including the β-adrenoceptor (Moret *et al.*, 1985). Repeated administration of milnacipran to rats at doses from 3 to 30 mg/kg/day did not modifiy the binding to β-adrenoceptors nor the activity of β-adrenergic agonist-induced stimulation of adenylate cyclase measured in the cerebral cortex. Twenty-one days treatment of rats with imipramine at 10 mg/kg/day, however, caused a reduction in the number of β-adrenoceptors without affecting the affinity for these receptors. It also decreased the stimulation of adenylate cyclase activity induced by the β-adrenergic agonist, isoprenaline, but not that induced by GTPγS or forskolin. These effects of imipramine were similar to those reported previously for imipramine (Duncan *et al.*, 1994) as well as those reported for desipramine (Sarai *et al.*, 1978; Bergstrom and Kellar, 1979; Riva and Creese, 1989).

The present study completes a series of investigations from several laboratories (Table 3) which collectively have studied doses ranging from 3 to 30 mg/kg/day for periods from 3 to 42 days. This range of doses and

Table 2. Effect of repeated administration of milnacipran and imipramine on β-receptor-linked adenylate cyclase activity in rat cortex

		Stimulating agent		
		Isoprenaline	GTPγS	Forskolin
		Percentage increase over basal values		
Control		18.8 ± 3.5	266.2 ± 32.5	1474 ± 194
Milnacipran	(3 mg/kg/day)	19.8 ± 3.5	280.5 ± 35.9	1468 ± 201
	(10 mg/kg/day)	17.2 ± 0.7	257.0 ± 30.8	1558 ± 198
	(30 mg/kg/day)	12.6 ± 4.3	245.0 ± 29.6	1462 ± 226
Imipramine	(10 mg/kg/day)	5.6 ± 2.9*	238.5 ± 29.7	1466 ± 210

Values are means ± SEM of six animals/group. * p <0.02 compared to the corresponding control values. Samples were stimulated by isoprenaline (30 μM), GTPγS (10 μM) or forskolin (10 μM).

Table 3. Summary of studies of β-adrenoceptors after repeated administration with milnacipran

Total daily dose (mg/kg)	Administrations per day	Route of administration	duration of administration	Reference	Parameters studied
3	2	intraperitoneal	21 days	present study	B,C
10	2	intraperitoneal	21 days	present study	B,C
15	2	intraperitoneal	14 days	Carré *et al.*, 1988	B
15	2	oral (gavage)	3 days	Assié *et al.*, 1992	B
15	2	intraperitoneal	21 days	Carré *et al.*, 1988	B
15	continuous	oral (drinking water)	42 days	Moret *et al.*, 1985	B
15	continuous	oral (drinking water)	42 days	Assié *et al.*, 1990	S
15	continuous	oral (drinking water)	42 days	Assié *et al.*, 1992	B
20	1	intraperitoneal	15 days	Matsubara *et al.*, 1988	B
20	1	intraperitoneal	15 days	Matsubara *et al.*, 1990	B,C
30	1	oral (gavage)	21 days	Assié *et al.*, 1992	B
30	2	intraperitoneal	21 days	present study	B,C
32	continuous	subcutaneous(osmotic mini-pumps)	27 days	Assié *et al.*, 1992	B

B = β-adrenoceptor binding, C = β-adrenoceptor-linked cyclase, S = salbutamol-induced hypoactivity.

duration of administration eliminates the possibility of an effect occurring only at a lower dose or a transient effect occurring after a few days only. The possibility that the relatively short half-life of milnacipran (7 hr in rats, unpublished data) could be responsible for the lack of effect has been eliminated by the use of twice daily injections, administration in the drinking water and continuous infusion from sub-cutaneous osmotic mini-pumps (Assié *et al.*, 1992). In addition the lack of an effect on adenylate cyclase activity (Matsubara *et al.*, 1990; present study) confirms that β-adrenoceptor function is not impaired without a modification in β-adrenoceptor binding. Furthermore, behavioural studies have shown that the β-adrenoceptor-linked behaviour, salbutamol-induced hypolocomotion in rats, was not modified by repeated administration of milnacipran (15 mg/kg/day for 6 weeks) (Assié *et al.*, 1990) whereas it was decreased following repeated administration of imipramine (15 mg/kg/day for 6 weeks).

In view of this series of investigations it is highly unlikely that the repeated administration of milnacipran, in contrast to tricyclic antidepressants, has any effect on the sensitivity of the β-adrenoceptor. Since milnacipran inhibits the reuptake of noradrenaline and increases the extracellular levels of noradrenaline *in vivo* as measured by microdialysis (Moret and Briley, 1996), it is rather surprising that there is no influence on β-adrenoceptor sensitivity. For the moment we have no clear explanation but the very low lipophilicity of milnacipran compared to tricyclic and many other antidepressants may be important. It is possible that the very high local concentration of the lipophilic antidepressants in the membranes surrounding β-adrenoceptors have effects that are not directly related to their activation of the receptor by increased noradrenaline levels.

Since milnacipran is a clinically effective antidepressant, β-adrenoceptor down-regulation would thus not appear to be implicated in its antidepressant activity. The initially proposed universality of β-adrenoceptor down-regulation by antidepressants has already been shown not to apply to all selective serotonin reuptake inhibitors (Hyttel *et al.*, 1984; Nelson *et al.*, 1989). The present results show that the phenomenon cannot even be extended to all noradrenaline reuptake inhibiting antidepressants. There is clearly no predictive value to the ability of a new antidepressant to down-regulate β-adrenoceptors in the cortex of normal rats. This does not, however, necessarily mean that β-adrenoceptor sensitivity changes do not play a part in the physiopathology of depression. This remains for the moment an open question.

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EXHIBIT 6

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Repeated administration of milnacipran induces rapid desensitization of somatodendritic 5-HT_{1A} autoreceptors but not postsynaptic 5-HT_{1A} receptors

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The effects of the repeated administration of milnacipran, a serotonin (5-HT)-noradrenaline reuptake inhibitor (SNRI), on the functional status of somatodendritic 5-HT_{1A} receptors, and postsynaptic 5-HT_{1A} receptors were explored using electrophysiological approaches in rats. In-vitro electrophysiological recordings in the dorsal raphe nucleus showed that 5-HT inhibited the firing of serotonergic neurones, and the selective 5-HT_{1A} receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane carboxamide (WAY 100635), reversed the inhibitory effect of 5-HT. The potency of 5-HT to inhibit the firing of serotonergic neurones was slightly attenuated after 3 days of treatment with milnacipran (30 mg/kg, p.o., twice daily), and significantly attenuated after 7 or 14 days treatment at the same dose. The tricyclic antidepressant, imipramine, did not significantly modify the inhibitory effect of 5-HT. After 7 days treatment at 30 mg/kg, p.o., once daily, milnacipran reduced the potency of 5-HT to inhibit the firing of serotonergic neurones, whereas the selective serotonin reuptake inhibitors, fluvoxamine and fluoxetine (60 and 30 mg/kg, p.o., once daily, respectively), did not modify it under these conditions. Treatment with milnacipran (30 mg/kg, p.o., twice daily) for 14 days did not change the inhibition of the CA1 field potential in rat hippocampal slices by 5-HT. These data suggest that somatodendritic 5-HT_{1A} receptors, but not postsynaptic 5-HT_{1A} receptors, rapidly desensitize in response to the repeated administration of milnacipran.

Key words: antidepressants, desensitization, dorsal raphe nucleus, 5-HT_{1A} autoreceptor, milnacipran, serotonin and noradrenaline reuptake inhibitor

Introduction

All known antidepressants take at least 2–3 weeks to manifest their antidepressant activity in humans although they exert their biochemical effects, such as the inhibition of monoamine (serotonin, 5-HT; noradrenaline, NA) uptake, within hours. The down-regulation theory has been proposed as a possible explanation for this discrepancy. In brief, the fact that most tricyclic antidepressants (TCAs) decrease the density of β-adrenoceptors and/or 5-HT_2 receptors after their repeated administration for 2–3 weeks has led to the idea that down-regulation of these receptors may be involved in the mechanism of onset of antidepressant activity (Peroutka and Snyder, 1980; Sulser *et al.*, 1983). However, it has become evident that some selective serotonin reuptake inhibitors (SSRIs) do not produce down-regulation of β-adrenoceptors or 5-HT_2 receptors (Hyttel *et al.*, 1984; Nelson *et al.*, 1990; Goodnough and Baker, 1994).

Milnacipran is a specific 5-HT and NA reuptake inhibitor (SNRI), with almost the same potency for inhibiting the sodium-dependent uptake of both 5-HT and NA into the rat cerebral cortex with no affinity for the receptors responsible for the adverse effects of TCAs (Moret *et al.*, 1985; Mochizuki *et al.*, 2002). Milnacipran increases extracellular levels of 5-HT and NA to the same degree in the rat prefrontal cortex (Mochizuki *et al.*, 2002) and guinea-pig hypothalamus (Moret and Briley, 1997). Repeated administration of milnacipran does not produce down-regulation of β-adrenoceptors or 5-HT_2 receptors (Assié *et al.*, 1992; Matsubara *et al.*, 1993; Neliat *et al.*, 1996) in spite of the fact that milnacipran is a clinically effective antidepressant (Kasper *et al.*, 1996; Lopez-Ibor *et al.*, 1996; Puech *et al.*, 1997). Moreover, it does not produce any change in the β-adrenoceptor-linked adenylate cyclase system in the rat cerebral cortex (Matsubara *et al.*, 1993; Neliat *et al.*, 1996). Therefore the mechanism of the onset of action of milnacipran cannot be explained by the down-regulation of these receptors.

Somatodendritic 5-HT_{1A} autoreceptors, which are located on

the cell bodies and dendrites of 5-HT neurones in the raphe nucleus, exert a negative feedback control on cell firing (Sprouse and Aghajanian, 1987; Jolas *et al.*, 1993). This results in a decrease in 5-HT turnover (Hamon *et al.*, 1988) and 5-HT release (Sharp *et al.*, 1989) in all brain areas to which these neurones project. Acute administration of SSRIs, such as citalopram and fluvoxamine, leads to a greater increase in the extracellular concentration of 5-HT in the raphe nuclei than in the nerve endings of the frontal cortex or hippocampus (Bel and Artigas, 1992; Invernizzi *et al.*, 1992; Celada and Artigas, 1993; Gartside *et al.*, 1995; Malagié *et al.*, 1995). The excess 5-HT in the extracellular space of the raphe nucleus activates 5-HT_{1A} autoreceptors in the soma and dendrites of serotonergic neurones and reduces the neuronal activity and release of 5-HT by nerve terminals in forebrain (for a review, see Artigas *et al.*, 1996). However, chronic administration of SSRIs for 14–28 days leads to functional desensitization of somatodendritic 5-HT_{1A} autoreceptors, with the result that 5-HT release is increased in the prefrontal cortex (Bel and Artigas, 1993). It has been suggested that the delayed onset in the clinical action of SSRIs might be explained by the time required for this desensitization (Blier and de Montigny, 1994; Gardier *et al.*, 1996).

Few reports have investigated the effect of chronic milnacipran administration on the serotonergic system. Milnacipran, given chronically, modified neither the release of [^{3}H]5-HT induced by electrical stimulation nor the inhibitory effect of the agonist, LSD, in rat hypothalamus slices (Moret and Briley, 1990). This result suggested that chronic treatment of milnacipran does not desensitize 5-HT_{1B} autoreceptors located on 5-HT terminals. On the other hand, Mongeau *et al.* (1998) demonstrated that the firing rate of the raphe 5-HT neurones is reduced following a 2-day treatment with milnacipran with a complete recovery after a 14-day treatment. Although the recovery was suggested to be attributed to a desensitization of the somatodendritic 5-HT_{1A} autoreceptors, the inhibitory effect of 5-HT and of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), on the firing rate of 5-HT neurones was unaltered after a 14-day treatment with milnacipran (Mongeau *et al.*, 1998). Thus, further studies are needed to clarify the effect of milnacipran on the sensitivity of somatodendritic 5-HT_{1A} autoreceptors.

To investigate whether somatodendritic 5-HT_{1A} autoreceptors are desensitized, we examined the effects of the repeated administration of milnacipran in rats on the concentration-dependent inhibition by 5-HT of firing in the dorsal raphe nucleus using in-vitro electrophysiological methods. The effects of repeated administration of milnacipran were compared to those of imipramine and the SSRIs, fluvoxamine or fluoxetine. In addition, we studied whether repeated administration of milnacipran modifies the function of postsynaptic 5-HT_{1A} receptors, by examining the effect of 5-HT on the concentration-dependent inhibition of CA1 field potential.

Materials and methods

Animals

These experiments were performed on male Wistar rats (Charles River, Japan). Rats, weighing 210–280 g and aged 7 weeks at the time of electrophysiology experiments, were housed in a room on a 12-h dark/light cycle at 22–25 °C with food and water provided *ad libitum*. All animal studies were approved by the Animal Care and Use Committee of the Institute for Life Science Research, Asahi Kasei Corporation and were in compliance with the Guide for the Care and Use of Laboratory Animals of the Institute for Life Science Research, Asahi Kasei Corporation.

Drugs

Milnacipran hydrochloride (synthesized by Pierre Fabre Médicament, Castres, France), imipramine hydrochloride (Sigma, St Louis, MO, USA), fluvoxamine maleate, fluoxetine hydrochloride and WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide) (synthesized by Asahi Kasei Corporation, Shizuoka, Japan) were used. All other compounds used were analytical grade and were obtained from Wako (Osaka, Japan), Sigma (St Louis, MO, USA) and Research Biochemical International (Natick, MA, USA). All drugs tested were dissolved in distilled water, and were administered p.o. in a volume of 0.2 ml per 100 g body weight.

Firing rate of dorsal raphe nucleus neurones in brain slices

In the study which compared milnacipran with imipramine, rats were orally administered twice daily for 3, 7 or 14 days with milnacipran (30 mg/kg), imipramine (30 mg/kg) or distilled water. Rats were anaesthetized with ether and decapitated 12 h after the last administration. In the study comparing milnacipran with SSRIs, rats were orally administered for 7 days with milnacipran (30 mg/kg), fluvoxamine (60 mg/kg), fluoxetine (30 mg/kg) or distilled water once daily, because of the long half-life of the SSRIs. Rats were anaesthetized with ether and decapitated 24 h after the last administration.

The brains were rapidly removed and immersed in ice-cold Krebs' solution, bubbled continuously with a 95% O_2/5% CO_2 mixture. Coronal sections (350 μm thick) were cut through the dorsal raphe nucleus using a vibratome. The slices were allowed to recover for 1.5 h at 34 °C in a submersion type slice chamber. The artificial cerebrospinal fluid (ACSF) contained (in mM): NaCl 126, KCl 5, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHPO_4$ 1.26, $NaHCO_3$ 26, glucose 10, pH 7.4, bubbled continuously with a 95% O_2/5% CO_2 mixture. The α_1-adrenoceptor agonist, phenylephrine (5 μM), was added to the ACSF to activate the serotonergic neurones because serotonergic neurones do not discharge spontaneously under these conditions. A single slice was transferred to a recording chamber in which it was continuously superfused with oxygenated ACSF (3 ml/min) at 34 °C.

Single-unit extracellular recordings were performed with a single barrel micropipette (tip diameter 1–2 μm) filled with 2 M NaCl. The micropipette was introduced into the dorsal raphe nucleus area until the activity of a neurone was detected on an oscilloscope. The electrode signal was amplified (MEZ-8301, Nihon Kohden, Japan) and monitored on the oscilloscope. Putative serotonergic neurones were identified on the basis of their duration, regular pattern of discharge and slow firing rate (Vandermaelen and Aghajanian, 1983). Integrated firing rate was computed in 10 s samples from an electronic counter (MET-1100, Nihon Kohden, Japan) triggered by individual neuronal spikes.

The percentage inhibition of firing was calculated by comparing the baseline firing rate to the response to 5-HT, which was a parameter of neuronal sensitivity to the 5-HT_{1A} autoreceptor (Schechter *et al.*, 1990; Jolas *et al.*, 1993; Le Poul *et al.*, 1999).

Single cells were considered to be 'desensitized' when the firing was not totally suppressed by 10 μM 5-HT. In general, firing in 'desensitized' cells was suppressed by 50% by 10 μM 5-HT.

Evoked potential of hippocampal CA1 neurones in brain slices

Rats were administrated orally twice daily for 14 days with milnacipran (30 mg/kg), imipramine (30 mg/kg) or distilled water. Rats were anaesthetized with ether and decapitated at least 12 h after the last administration. Hippocampal slices were cut and treated with the same procedure as dorsal raphe nucleus slices, except for the cutting area and thickness (400 μm). Evoked potentials were recorded from the pyramidal layer of the CA1 region with a glass micropipette (tip diameter 30–50 μm) filled with 2 M NaCl. Schaffer collateral-commissural fibre pathways were stimulated by constant current pulses (0.1 ms duration, 0.33 Hz frequency). Electrode signals were amplified and monitored by the system described above. The micropipette was introduced into the CA1 area until the activity of neurones was detected. The intensity of the stimulus was adjusted to obtain maximal amplitude. Mean amplitude of field potential from eight samples was measured using the MacLab system (AD Instruments, Castle Hill, Australia).

The percentage inhibition by 5-HT was calculated by comparing the baseline amplitude of the response to 5-HT, which was a parameter of neuronal sensitivity of the postsynaptic 5-HT_{1A} receptor (Maj *et al.*, 1996).

Statistical analysis

Data were analysed by analysis of variance (ANOVA) with repeated measures. When significant differences were found, post-hoc comparisons were made with Dunnett's test.

Results

Effects of 5-HT and WAY 100635 on the firing rate of dorsal raphe nucleus serotonergic neurones

Bath application of 5-HT (1–10 μM) to the brain stem slices resulted in a concentration-dependent reduction in the firing rate of dorsal raphe nucleus serotonergic neurones (data not shown). Firing was completely abolished by the application of 10 μM 5-HT, and this inhibition was reversed by the selective 5-HT_{1A} receptor antagonist, WAY 100635 (10–30 nM) (Fig. 1).

Effect of repeated administration with milnacipran or imipramine on 5-HT-induced inhibition of the firing rate of dorsal raphe nucleus serotonergic neurones

Baseline firing activity in the dorsal raphe nucleus was unchanged after repeated administration (30 mg/kg) of milnacipran or imipramine for 3, 7 or 14 days compared to control. Individual firing rates of the neurones ranged from 8.9–22.7 spikes/10 s and the mean rates of baseline firing of each group varied from 12.2–16.1 spikes/10 s. Superfusion with ACSF containing 5-HT (1–10 μM) produced a concentration-dependent inhibition of the dorsal raphe nucleus firing rate, with a complete inhibition at a concentration of 10 μM, in each control group treated for 3, 7 or 14 days (data not shown). In the group treated with milnacipran

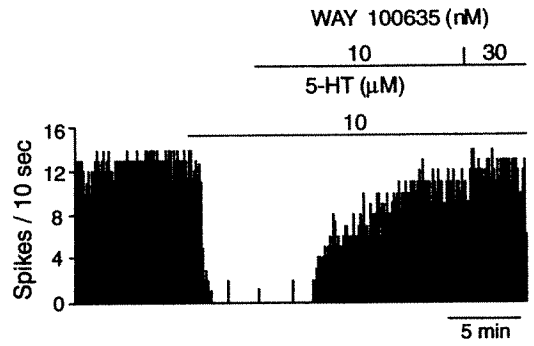

Figure 1 Antagonism by WAY 100635 of the inhibitory effect of 5-HT on the firing of dorsal raphe nucleus serotonergic neurones in brain stem slices. The integrated firing rate record shows the typical response of the dorsal raphe nucleus neurones to drugs, which were applied (horizontal bar) with perfusate at the concentrations indicated

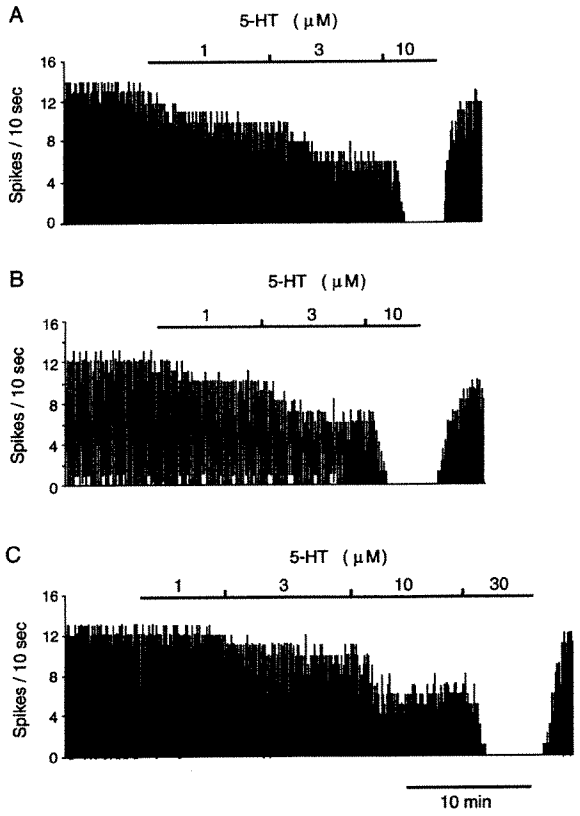

Figure 2 Integrated firing rate records of typical responses of dorsal raphe nucleus neurones to 5-HT in brain stem slices from rats treated for 7 days with distilled water (control, A), impramine at 30mg/kg, p.o., twice daily (B) or milnacipran at 30mg/kg p.o., twice daily (C). The desensitization of 5-HT_{1A} autoreceptors was determined as the decrease of the response to 5-HT, and the baseline rate of neurones used for 5-HT responses was > 10 spikes/10 s. 5-HT was perfused, at the concentrations indicated, until the response was stable (at least 5 min) at each concentration

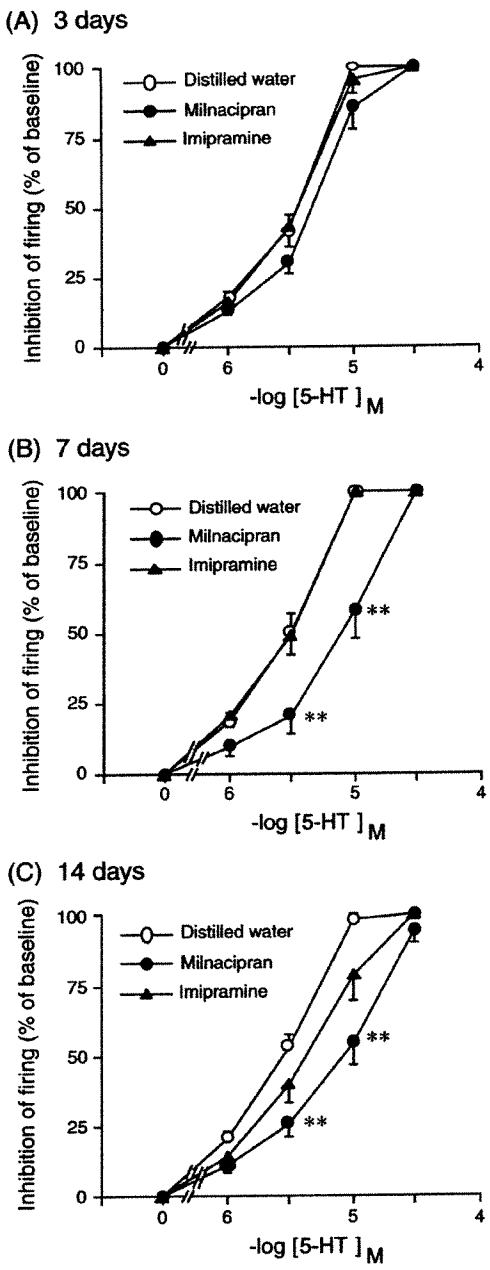

Figure 3 Effect of a repeated administration (30 mg/kg p.o., twice daily) of milnacipran and imipramine for 3 days (A), 7 days (B) or 14 days (C) on the concentration-dependent inhibition of the dorsal raphe nucleus firing by 5-HT in rat brain stem slices. The ordinate is expressed as percentage of baseline firing rate before perfusion with 5-HT. Each point represents the mean ± SEM of data obtained from all recorded neurones (n = 10, one cell per rat). $^{**}p < 0.01$ compared to water-treated group

(30 mg/kg p.o., twice daily) for 3, 7 or 14 days, the inhibitory effect by 10 μM 5-HT was partially attenuated (approximately 50% inhibition) in 30%, 80% and 90%, respectively, of all recorded cells. After repeated treatment with imipramine

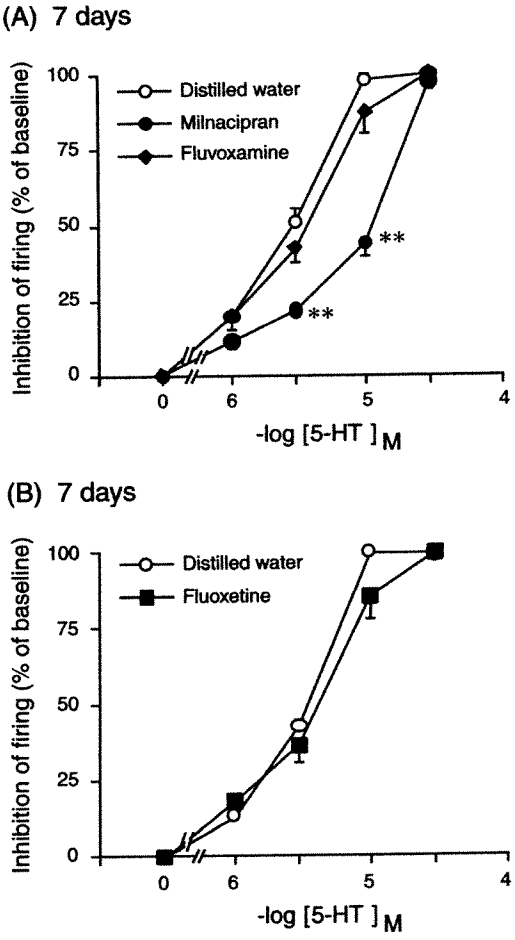

Figure 4 Effect of a repeated administration of milnacipran (30 mg/kg, p.o., once daily) (A) and fluvoxamine (60 mg/kg, p.o., once daily) (A) or fluoxetine (30 mg/kg, p.o., once daily) (B) for 7 days on the concentration-dependent inhibition of the dorsal raphe nucleus firing by 5-HT in rat brain stem slices. The ordinate is expressed as percentage of baseline firing rate before perfusion with 5-HT. Each point represents the mean ± SEM of data obtained from all recorded neurones (n = 10, one cell per rat). $^{**}p < 0.01$ compared to water-treated group

(30 mg/kg p.o., twice daily) for 14 days, but not 3 or 7 days, the reduced inhibition by 5-HT was observed in approximately 50% of all cells tested. Figure 2 shows typical firing rate responses to 5-HT in each treatment group after 7 days of administration.

The 5-HT-induced inhibition, obtained from all tested cells, was slightly attenuated after 3 days of treatment with milnacipran (30 mg/kg p.o., twice daily), and significantly attenuated after 7 and 14 days treatment (Fig. 3). Inhibition of firing by 10 μM 5-HT was 82% (not significantly different from controls), 58% ($p < 0.01$) and 54% ($p < 0.01$), respectively, after treatment with milnacipran for 3, 7 and 14 days (Fig. 3). The firing was completely inhibited in all cells measured in the control group (Fig. 3). The concentration inhibition curve of 5-HT was shifted to the right in rats treated with milnacipran for 7 and 14 days (Fig. 3). Treatment with imipramine (30 mg/kg p.o., twice daily) for 3 and 7 days resulted in no

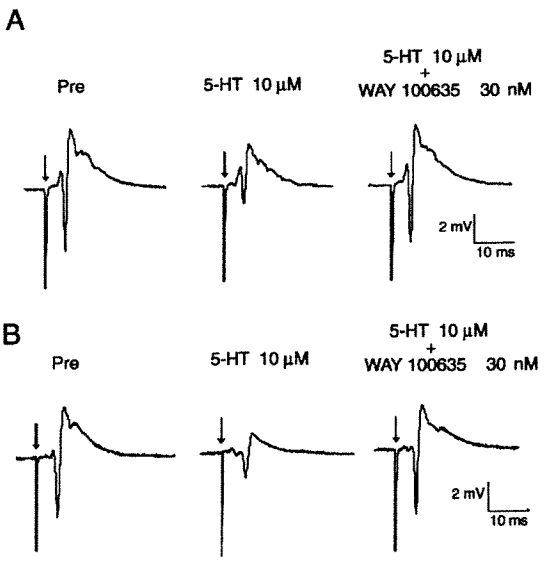

Figure 5 CA1 field potential records of typical responses to 5-HT in hippocampal slices from rats treated with distilled water (A) or milnacipran at 30 mg/kg p.o., twice daily for 14 days (B). Field potentials evoked by electrical stimulation (arrow) of the Schaffer collateral-commissural fibre pathway were recorded from the pyramidal layer of CA1 before the application of 5-HT (Pre), after 8 min in the presence of 10 μM 5-HT and a further 8 min in the presence of 5-HT and 30 nM WAY 100635

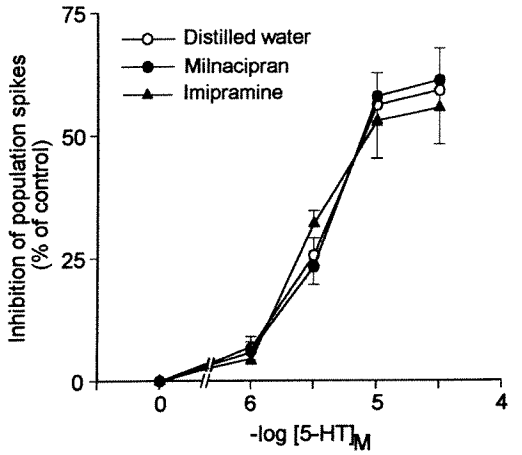

Figure 6 Effect of a repeated administration (30 mg/kg p.o., twice daily) of milnacipran and imipramine for 14 days on the concentration-dependent inhibition of CA1 field potential by 5-HT in rat hippocampal slices. 5-HT was perfused for 8 min at each concentration. The ordinate is expressed as percentage of the amplitude before the perfusion with 5-HT. Each point represents the mean ± SEM of data obtained from all recorded slices (n = 10, one cell per rat)

significant change in the 5-HT-induced inhibition of firing. There was a small but not significant attenuation of the firing inhibition induced by 5-HT after 14 days of treatment with imipramine (Fig. 3).

Effect of repeated treatment with milnacipran or SSRIs on 5-HT-induced inhibition of the firing rate of dorsal raphe nucleus serotonergic neurones

The baseline firing activity in the dorsal raphe nucleus was unchanged by repeated once daily administration of milnacipran, fluvoxamine or fluoxetine for 7 days compared to control. Individual firing rates of the neurones ranged from 10.4–24.0 spikes/10 s and the mean rates of baseline firing of each group varied from 15.6–18.3 spikes/10 s. Following repeated treatment with milnacipran (30 mg/kg p.o., once daily) for 7 days, sensitivity to 10 μM 5-HT was reduced in all recorded cells (10/10). Repeated administration of fluvoxamine (60 mg/kg p.o., once daily) and fluoxetine (30 mg/kg p.o., once daily) for 7 days caused a decrease of the sensitivity to 5-HT in 30% of all tested cells (3/10 for each compound).

The 5-HT-induced inhibition of firing after 7 days treatment with milnacipran was significantly attenuated to a similar extent as the previous experiment comparing milnacipran with imipramine (Fig. 4). Treatment with fluvoxamine or fluoxetine for 7 days had no effect on the 5-HT-induced inhibition of firing (Fig. 4).

Effect of repeated treatment with milnacipran or imipramine on the evoked potential in hippocampal pyramidal cells

Bath application of 5-HT (1–10 μM) to the hippocampal slices resulted in a concentration-dependent reduction of the amplitude of population spikes evoked in the CA1 pyramidal neurones by electrical stimulation of the Schaffer collateral-commissural fibre pathway (data not shown). The amplitude of population spikes was decreased approximately 50–60% by the application of 10 μM 5-HT, and this inhibition was reversed by WAY 100635 (30 nM) (Fig. 5).

The mean amplitude of population spikes evoked in CA1 pyramidal cells before the application of 5-HT, 7.0 and 6.5 mV, was unchanged by repeated administration of distilled water or milnacipran for 14 days, respectively. The 5-HT-induced inhibition was not altered by the 14-day-treatment with milnacipran (Figs 5 and 6) or imipramine (Fig. 6).

Discussion

The present electrophysiological study demonstrates the effects of repeated administration of milnacipran on the adaptation of the serotonergic system. It is well known that in-vitro perfusion of brain stem slices with 5-HT (Vandermaelen and Aghajanian, 1983) or 5-HT_{1A} receptor agonists or partial agonists, such as 8-OH-DPAT, buspirone and ipsapirone (Jolas *et al.*, 1993; Vandermaelen *et al.*, 1986), inhibits the firing rate of dorsal raphe serotonergic neurones, and this inhibitory action is antagonized by a 5-HT_{1A} receptor antagonist (Sprouse and Aghajanian, 1986; Corradetti *et al.*, 1996). In agreement with these reports, the inhibitory effect of 5-HT was antagonized by the application of WAY 100635, a selective 5-HT_{1A} receptor antagonist. Thus, the inhibitory action of

5-HT observed in our studies occurs via the stimulation of somatodendritic 5-HT_{1A} autoreceptors. This inhibitory effect of 5-HT was significantly attenuated by repeated treatments with milnacipran for 7 and 14 days (but not 3 days). Consequently, the somatodendritic 5-HT_{1A} autoreceptors were desensitized by repeated treatment with milnacipran after only 7 days.

Mongeau *et al.* (1998) reported that milnacipran treatment (60 mg/kg, s.c. for 14 days) did not significantly desensitize the somatodendritic 5-HT_{1A} autoreceptors, although the response to 5-HT and 8-OH-DPAT on the firing rate of raphe serotonergic neurones showed a tendency to decrease. The route of administration of the drug might be a major source of discrepancy between the study by Mongeau *et al.* (1998) and the present work. In their studies, milnacipran was administered using subcutaneous osmotic minipumps, whereas in the present study, milnacipran was treated orally. Although osmotic minipumps implanted subcutaneously are expected to deliver more drug to the brain since the first-pass liver elimination is avoided, they do so at a low constant rate. It has been shown that when [^{14}C]milnacipran (5 mg/kg, p.o.) was administered to rats, the radioactivity was relatively low in the central nervous system (Sakai *et al.*, 1994) because of the lipophobic nature of the molecule. It is possible that the administration of milnacipran with osmotic minipumps to rats might not result in plasma levels sufficient to obtain significant entry into the brain, whereas the peak plasma concentrations following an oral bolus dose would be considerably higher. This point obviously requires further investigation. In fact, milnacipran treatment by osmotic minipumps for 2 days did not inhibit [^{3}H]5-HT uptake in rat brain slices in ex-vivo experiments (Mongeau *et al.*, 1998), which is in contrast to results showing that orally administered milnacipran inhibits the reuptake of 5-HT *in vivo* (Moret *et al.*, 1985). Bel and Artigas (1999) have also studied the effect of a 2-week treatment with milnacipran on the baseline extracellular levels of 5-HT measured in frontal cortex and raphe nuclei by microdialysis. As in the study by Mongeau *et al.*, milnacipran was administered subcutaneously using osmotic minipumps (30 and 60 mg/kg per day). The output of 5-HT was unaffected by this prolonged treatment. This result, which suggests an absence of any change of sensitization of receptors implicated in the control of 5-HT output after minipump application of milnacipran, appears to be consistent with the data of Mongeau *et al.* (1998). However, further study of the sensitivity of the 5-HT_{1A} autoreceptor using a selective agonist would add support to this conclusion.

Another methodological difference between the study of Mongeau *et al.* (1998) and the present study is the fact that Mongeau *et al.* tested for desensitization *in vivo* rather than in brain slices. In the present work, serotonergic neurones were isolated from their endogenous noradrenergic input and activated with an exogenous α_1-adrenergic agonist, and this may be important for studying 5-HT_{1A} autoreceptor desensitization. Because serotonergic neurones do not discharge spontaneously *in vitro*, the use of an α_1-adrenoceptor agonist is necessary, whereas they discharge spontaneously *in vivo*. A concentration of 5 μM was required to obtain a sufficient number of cells firing at over 10 spikes/10 s. In our studies, individual firing rates of the neurones ranged from 8.9–24.0 spikes/10 s. The mean rates of baseline firing varied from 12.2–16.1 spikes/10 s in the milnacipran/imipramine study and 15.6–18.3 spikes/10 s in the milnacipran/SSRIs study. Furthermore, Hensler *et al.* (1996) have shown that 5-HT_{1A} and α_1-adrenergic receptors may be coupled to the same effectors. This point should be taken into account for the difference between the in-vitro and in-vivo studies. The interpretation of the in-vivo data may also be complicated by the fact that milnacipran is a powerful noradrenergic reuptake inhibitor (Briley *et al.*, 1996). This noradrenergic influence may be important in the control of 5-HT activity (Mongeau *et al.*, 1998).

In contrast to milnacipran, repeated treatment with imipramine for 3 or 7 days did not influence the inhibitory effect of 5-HT but, after 14 days, there was a non-significant tendency to attenuate it. A statistically significant desensitization of the somatodendritic 5-HT_{1A} autoreceptors might have been observed if imipramine had been administered for a longer time. Consistent with the present study, Blier and de Montigny (1980) have also shown that imipramine does not desensitize the somatodendritic 5-HT_{1A} autoreceptors after 14 days of treatment. These studies suggest that milnacipran may result in a more rapid desensitization than imipramine. Interestingly, an early open clinical study in Europe (Serre *et al.*, 1986) and a double-blind clinical comparative trial in Japan (Matsubara *et al.*, 1995) both suggested that milnacipran may have a more rapid onset of clinical action than imipramine with an effect after 1 week. Other trials gave no such indication (Puech *et al.*, 1997), although the trials were not specifically designed to study the onset of action. Clearly, further clinical trials are required to clarify this point.

In the current study, repeated treatment with either fluvoxamine or fluoxetine for 7 days did not significantly modify the sensitivity of the somatodendritic 5-HT_{1A} autoreceptors. This is in agreement with previous studies (Blier and de Montigny, 1994) where desensitization occured only after 2 weeks. In a study similar to the present one carried out *ex vivo* in rat brain slices with fluoxetine and paroxetine injected daily at the dose of 5 mg/kg, i.p., somatodendritic 5-HT_{1A} autoreceptors were shown to be significantly desensitized by the two SSRIs after 3 days treatment (Le Poul *et al.*, 1995). Although our experimental conditions are somewhat different from theirs (doses and route of administration), the two studies are basically in agreement. In the present study, desensitization was found in 30% of all tested neurones after 7 days with fluvoxamine or fluoxetine (compared to 50% of all tested neurones in the study by Le Poul *et al.*) and in 80–100% of all tested neurones after 7 days with milnacipran. A possible cause of the slightly increased desensitization with the SSRIs in the study by Le Poul *et al.* may have been the use of the 5-HT_{1A} autoreceptor agonist, 8-OH-DPAT, whereas 5-HT was used in the present study. Le Poul *et al.* used 8-OH-DPAT at subsaturating nanomolar concentrations, whereas 5-HT was used at saturating 1–10 μM concentrations in the present work. Because of its lower concentration, 8-OHDPAT is likely to be more sensitive to early stages of 5-HT_{1A} receptor desensitization which may explain the slightly greater proportion of desensitized neurones reaching significance in their study but not in ours. A similar proportion of desensitized neurones (80–100% for milnacipran in the present study and 83% for paroxetine in the study by Le Poul *et al.*) was found after 7 days and 21 days, respectively, suggesting that both compounds have a similar efficacy but that milnacipran desensitizes more rapidly. As observed by Le Poul *et al.* (1995), we also obtained a progressive increase in the proportion of 5-HT cells with desensitized somatodendritic 5-HT_{1A} autoreceptors and we concur with their suggestion that this effect may be related to the slow development of antidepressant action.

Thus, in the present study, milnacipran, but not imipramine, fluvoxamine or fluoxetine, caused a desensitization of the somatodendritic 5-HT_{1A} autoreceptors at 7 days. It is possible that the reason for this lies in the effect of milnacipran on noradrenergic neurotransmission. Noradrenaline, through stimulation of α_1-adrenoceptors (Baraban and Aghajanian, 1980), increases the firing of raphe serotonergic neurones and may therefore influence the sensitivity of 5-HT_{1A} receptors on the same neurones, although the mechanism remains to be elucidated. Milnacipran is unique among the compounds tested in having an indirect stimulatory action on the α_1-adrenoceptors (through NA uptake blockade). Although imipramine increases NA release through the inhibition of reuptake, it is also a potent α_1-adrenoceptor antagonist (Doggrell and Vincent, 1981). The SSRIs, of course, have no direct short-term influence on noradrenergic firing activity.

Many investigators have suggested that the activation of postsynaptic 5-HT_{1A} receptors may be responsible for the therapeutic efficacy of SSRIs and 5-HT_{1A} receptor agonists (Bergeron *et al.*, 1995; De Vry, 1996; Berendsen and Broekkamp, 1997; Blier *et al.*, 1997). 5-HT decreased the amplitude of population spikes and this effect was blocked by the selective 5-HT_{1A} receptor antagonist, WAY 100635, consistent with a previous study showing that the inhibitory effect of 5-HT on the population spikes is mediated by postsynaptic 5-HT_{1A} receptors (Bijak *et al.*, 1996). Repeated administration of milnacipran for 14 days did not effect the neuronal responsiveness of hippocampal slices to 5-HT. Similar results were obtained after a treatment with imipramine for 14 days. These findings suggest that the repeated oral administration of milnacipran induces no functional desensitization in the postsynaptic 5-HT_{1A} receptors. Thus, milnacipran, similar to many SSRIs and 5-HT_{1A} receptor agonists, appears to induce a selective desensitization of the somatodendritic 5-HT_{1A} autoreceptors (Blier and de Montigny, 1983; Chaput *et al.*, 1986; Blier and de Montigny, 1987; Jolas *et al.*, 1993) without modifying the sensitivity of the postsynaptic 5-HT_{1A} receptors in the hippocampus.

In summary, the present study shows that repeated treatment with milnacipran produces a desensitization of somatodendritic 5-HT_{1A} autoreceptors after treatment for 7 days without any change in the function of postsynaptic 5-HT_{1A} receptors. If the rate of desensitization of somatodendritic 5-HT_{1A} autoreceptors is an important factor in the onset of antidepressant action, as has been suggested (Blier and de Montigny, 1994; Le Poul *et al.*, 1995; Artigas *et al.*, 2001), the present findings suggest that milnacipran may have a more rapid onset of action than some other antidepressants.

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EXHIBIT 7

Appendix E: Additional data provided by Collegium with respect to a pulsatile milnacipran formulation

Example 1: Preparation of an Alternative Immediate Release Milnacipran Tablet

The ingredients, manufacturing process, and tablet parameters for the immediate release portion of an alternative pulsatile release milnacipran formulation, referred to as Lot #5, are described below.

Ingredient	**Quantity per tablet, mg**	**Quantity per 7,000 tablets, g**
Milnacipran HCl	50.00	350
Microcrystalline Cellulose (Avicel® PH 102)	10.00	70
Pre-gelatinized Starch (Starch® 1500)	10.00	70
Purified Water	QS	
Magnesium Stearate	0.7	4.9

The following manufacturing procedure was used for the preparation of a Pilot Scale batch of Immediate Release Milnacipran tablets (Lot# 5):

Step 1: Sifting

Milnacipran hydrochloride, Avicel® PH102, and Starch® 1500 were sifted through a #40 mesh screen.

Step 2: Dry mixing

The sifted material from Step 1 was loaded into a V-cone blender and mixed for 10 minutes without an intensifier bar.

Step 3: Granulation

The dry mix from step 2 was granulated with purified water in a planetary mixer and the resulting wet mass was passed through a #12 mesh screen.

Step 4: Drying

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The wet mass from step 3 was dried in a tray oven at 50°C until the moisture content was below 1%.

Step 5: Milling

The dried granules from step 4 were passed through #30 mesh screen.

Step 6: Lubrication

Magnesium stearate was sifted through a #40 mesh screen and added to the granules obtained in Step 5. The mixture was mixed in a V-Cone blender for 5 minutes without an intensifier bar.

Step 7: Compression

The blend from step 6 was compressed into tablets with average tablet weight of 70.7 mg using a 4.76 mm round standard concave punch at a hardness of 6-8 kP.

Tablet parameter	**Lot# 5**
Weight (mg)	70 - 72
Thickness (mm)	4.13 - 4.18
Friability (%)	0.113
Disintegration time in water	5 minutes 5 seconds

Example 2: Pilot scale preparation of a Delayed Release Portion of Pulsatile Release Milnacipran Formulation

Lot# 5 immediate release tablets were used for preparation of a delayed release dosage form. The manufacturing procedure consisted of spraying an isopropyl alcohol based coating suspension onto the immediate release tablets fluidized in the fluid bed processor. Tablets were collected with the following coat weight gain: 10% (Lot# 6), 15% (Lot# 7), and 20% (Lot# 8). After the coating process was completed, the tablets were incubated ("cured") at 40°C for 2 hours in the oven drier. The ingredients and preparation procedure of isopropyl alcohol-based coating suspension are given below.

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Ingredients of isopropyl alcohol based coating suspension (Eudragit® L 100/S 100 blended in a ratio of 1 to 3 (w/w) L 100 to S 100).

Ingredient	**Quantity per batch, g**
Eudragit L 100	15
Eudragit S 100	45
Isopropyl alcohol	854
Purified water	50
Triethyl citrate	6
Talc	30

Suspension preparation procedure :

Eudragit® L 100 and Eudragit® S 100 were weighed and added to Isopropyl alcohol with stirring. Purified water was added to the solution. The solution was stirred until it became clear and triethyl citrate was added to the solution. The solution was stirred for 30 minutes at room temperature. Talc was added to the solution to form a suspension and the suspension was stirred for 5 minutes. The suspension was continually stirred during the coating process.

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The following coating parameters were used:

Coating equipment	Fluid bed processor
Blower speed	2100 rpm
Spray rate	3 g / min
Inlet temp.	38°C
Tablet bed temp	36°C
Exhaust temp.	33°C
Spray	Continuous spray
Pressure	2 kg / cm^2

Tablets with 10% coat weight gain (Lot# 6) had the following parameters: 75 – 80 mg weight, 4.73 – 4.76 mm diameter, 4.18 – 4.31 mm thickness, and 7-9 kP hardness.

In vitro drug release studies were conducted using a USP dissolution apparatus II (paddles) at 50 rpm. Experiments were conducted in dissolution media at a temperature of 37.0±0.5°C, first for 2 hours in 0.1 N hydrochloric acid, followed by 5 hours in pH 6.8 phosphate buffer, and then 4 hours in pH 7.0 phosphate buffer. Samples were periodically withdrawn and analyzed for milnacipran content using HPLC. The dissolution results for Lots# 6, 7, and 8 are shown in Figure 1.

Example 3: Pharmacokinetic Parameters of Delayed Released Milnacipran tablet (Lot# 6) in Healthy Human Volunteers

The milnacipran delayed release tablet (10% coating weight gain, Lot# 6) described in Example 2 was tested in a single dose one way 6-patient pilot bioavailability study under fasting conditions.

The average milnacipran plasma concentration for five subjects as a function of time after tablet administration is shown in Figure 2. Average pharmacokinetic parameters were obtained by determining the pharmacokinetic parameters for each individual study subject and subsequently averaging the values obtained. The calculated pharmacokinetic parameters were as follows: T_{max} is 7±2 hours, C_{max} is 100±20 ng/ml, AUC (0-24) is 1043±218 ng hr/ml, and AUC (0-inf) is 1303±304 ng hr/ml (Note that the

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data for five subjects were used to calculate the values above. The data for the 6th subject were not taken into account due to unexplainably low observed milnacipran plasma levels).

An IR milnacipran formulation was previously tested under fed conditions and it was found that the administration of 50 mg Milnacipran HCl capsule BID resulted in an AUC (0-24) equal to 2592 ng hr/ml, and an AUC (0-inf) equal to 2743 ng hr/ml. Although no direct comparison can be made with the data obtained in the current study due to different study conditions (fasting vs. fed), the AUC for 50 mg DR tablet given QD is essentially one half of that for 50 mg IR BID. This fact indicates that essentially all milnacipran released from DR tablet was absorbed in the GI tract despite the fact that drug release was delayed for several hours.

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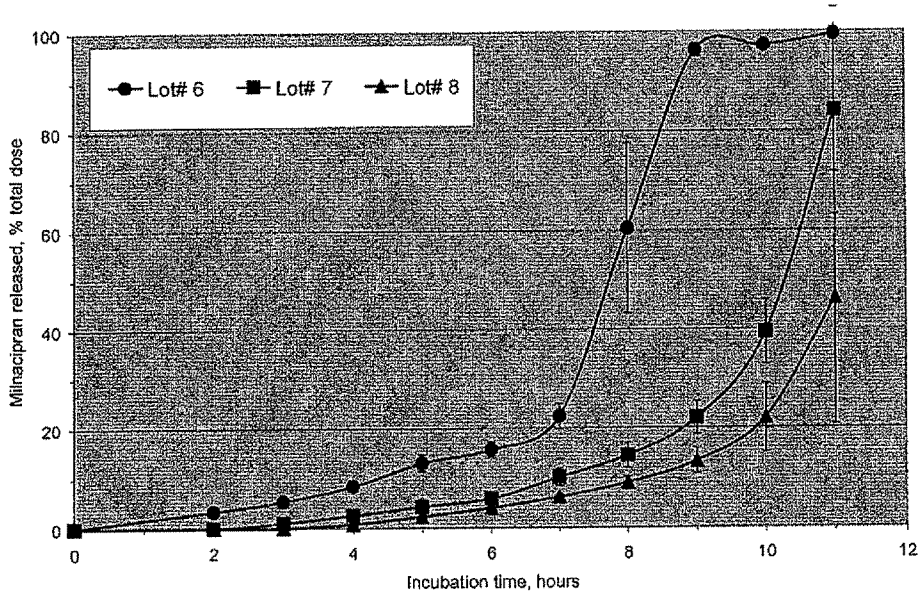

Figure 1. *In vitro* dissolution results for 50 mg milnacipran HCl delayed release tablet.

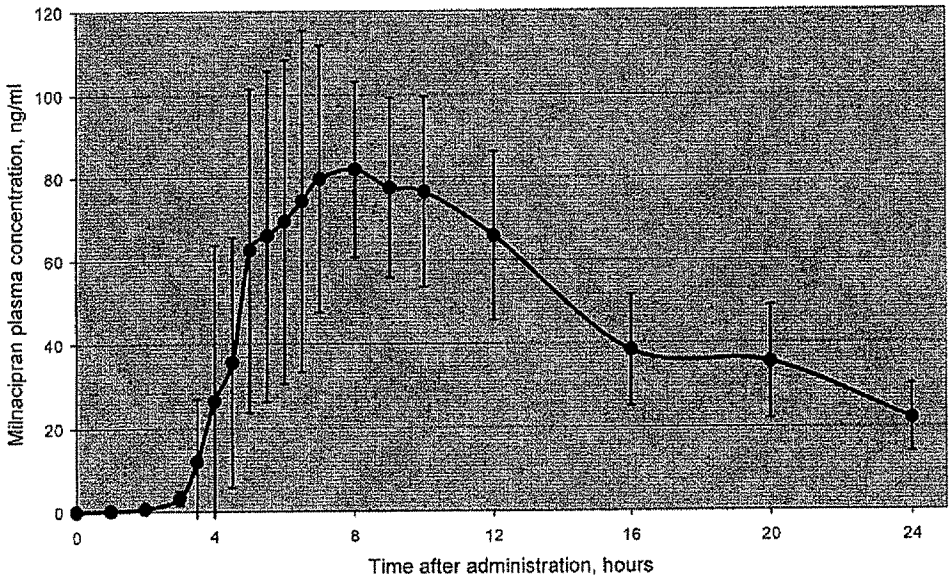

Figure 2. Pharmacokinetics of Delayed Released Milnacipran tablet in Healthy Human Volunteers.